JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXVI WASHINGTON, D. C., DECEMBER 1, 1923 NO. 9

THE QUANTITATIVE DETERMINATION OF CAROTIN BY MEANS OF THE SPECTROPHOTOMETER AND THE COLORIMETER[1]

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INTRODUCTION

The present status of our knowledge regarding carotin, as well as the other chloroplast pigments, is far from satisfactory. Due to this fact the various methods of quantitatively determining these pigments have been frequently investigated. Of the four chloroplast pigments, carotin, xanthophyll, chlorophyll a, and chlorophyll b, carotin is perhaps the best known, consequently more attempts have been made to determine this pigment quantitatively. In this paper, only methods which relate to carotin will be discussed, leaving the methods for xanthophyll and chlorophyll to be brought up later. Since the same principle is involved in quantitatively determining yellow pigments other than the carotinoids, the literature regarding these will be referred to briefly.[2]

In 1887 Arnaud (*1*) made a rough estimation of carotin by means of the colorimeter. This method was later used by Kohl, Willstätter and Stoll, Goerrig, Palmer (*12*), and Escher (*2*).

Monteverde and Lubimenko (*11*) devised a spectro-colorimetric method for quantitatively determining carotin, xanthophyll, and the chlorophylls. The method consisted in comparing the unknown with a standard so that the absorption spectra of the two solutions were of equal intensity.

Jacobson (*5*) determined the ether-soluble yellow pigments in alfalfa leaves by weighing the residue after saponification and evaporation of the solvents. This residue consisted not only of carotin but xanthophyll and ether-soluble impurities.

In 1912, Wurmser and Duclaux (*13*) spectrophotometrically determined the lipo-chrome (carotin and xanthophyll) content of *Chondrus crispus* and *Rhodymenia palmata*. Their measurements were made at $\lambda = 450$ millimicrons ($m\mu$) but they do not explain how the method was used.

Mathewson (1916) (*7*) has shown very conclusively that the spectrophotometer may be used in the analysis of food coloring matter. He (*8*) has given a procedure for estimating small amounts of organic compounds by converting them into colored derivatives. In a subsequent paper (*9*) spectrophotometric data which have been applied to the quantitative estimation of yellow AB, yellow OB, Butter Yellow and Sudan I are given.

Perhaps the greatest stride forward, in the matter of quantitative spectrophotometrical data regarding dyes has been made by Gibson, McNicholas, Tyndall, Frehafer, and Mathewson (*3*). They have given the data in a form which is readily available for analytical purposes.

[1] Accepted for publication October 2, 1923.

[2] Reference is made by number (italic) to Literature cited, p. 400.

From this review of the literature it is apparent that no accurate method for the estimation of carotin is readily available. Consequently, a rapid, accurate method for the determination of carotin by means of the spectrophotometer has been devised.[3, 4]

THE KÖNIG-MARTENS SPECTROPHOTOMETER

DEFINITION OF TERMS

The manner of using the instrument (*6*) in color analysis is not new. A more recent discussion of the method and apparatus is to be found in a Bureau of Standards Scientific Paper (*3*), No. 440. In order that this paper may be better understood by those who are not yet familiar with the König-Martens Spectrophotometer, a brief explanation of the spectrophotometric terms used here will be given.

When white light enters a column of colored solution the lights of different wave lengths are transmitted in unequal amounts. The blue to violet end of the spectrum is absorbed to a very great extent when light passes through a solution of carotin. In strong solutions all of the light is absorbed in this region (wave lengths of less than 500 millimicrons) and consequently none is transmitted. With a spectrophotometer it is possible to measure for any wave length the relative amount of light transmitted by a cell containing a solution, that is, the ratio

$$\frac{\text{light passing through last surface of cell}}{\text{light incident on first surface of cell.}}$$

This ratio is called the *transmission* (*T*) for this wave length.[5] The *Transmittance* (T) of the column of colored solution is the ratio of the light arriving at the second colored-solution-glass surface to the light passing the first colored-solution-glass surface, i. e., this ratio refers to the liquid in the cell only.

Further, the transmission of a given cell containing the solution is designated as (Sol *T*) while the transmission of the same or a duplicate cell containing pure solvent is designated as (Sov *T*). Then,

$$\frac{(\text{Sol } T)}{(\text{Sov } T)} = \frac{(\text{Sol } \mathsf{T})}{(\text{Sov } \mathsf{T})} \equiv \mathsf{T} \equiv \textit{Transmittancy},$$

which is thus defined as the ratio of that fraction of the incident radiant energy which is transmitted by a cell containing the solution, to that fraction of incident radiant energy which is transmitted by the same or a duplicate cell containing the solvent. This transmittancy (T) is the quantity which is obtained from the measurements made on the König-Martens instrument.

The *Specific Transmissivity* $\equiv \sqrt[bc]{\mathsf{T}} \equiv t$, where $b \equiv$ thickness (centimeters) of the layer of solution and $c \equiv$ concentration (centigrams per liter) of the dissolved substance.

The Specific Transmissive Index (others designate it as extinction coefficient or absorption index) $\equiv k \equiv -\log_{10} t = -\frac{1}{bc} \log_{10} \mathsf{T}$, i. e., $bck \equiv -\log_{10} \mathsf{T}$.

[3] All spectrophotometric data given in this paper were obtained on the König-Martens spectrophotometer at the Bureau of Standards.

[4] It is possible to use this method without making use of a spectrophotometer. A photometer, a mercury lamp and a filter to isolate line 435.8 are all that are necessary. See Bureau of Standards Tech. paper No. 119 (*4*).

[5] The following symbols, terms and definitions are in accordance with those defined in the Preliminary Report of the Optical Society of America, Committee on Nomenclature and Standards of Colorimetry, Irwin G. Priest, Chairman. 1919. (Unpublished. A photostat copy may be consulted in the Bureau of Standards Library.)

In all of the spectrophotometric work presented in this paper, the transmission of a cell containing the solution is always compared with that of a duplicate cell containing the solvent, hence, the quantity obtained from actual observations on a solution of given thickness and concentration is the transmittancy.

$-\text{Log}_{10}$ transmittancy, designated as $(-\log_{10}T)$ is identical with the product of thickness b, concentration c, and the specific transmissive index, k, and is represented by the term bck. For unit concentration and thickness, $-\log_{10}$ transmittancy becomes identical with specific transmissive index, which is the characteristic quantity, determined for any solution at a definite wave length or frequency.

In Figure 1 the concentration of pigment in centigrams per liter (at top) can be read on the X-axis. On the right hand side of the graph

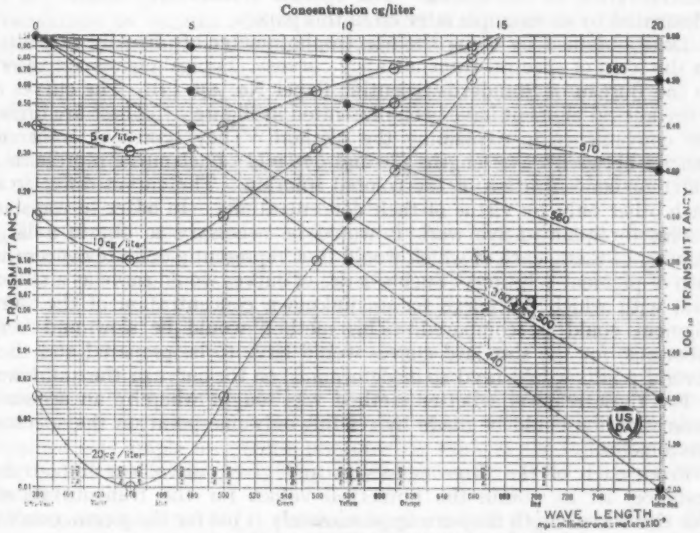

FIG. 1.—Spectral transmittancy of a theoretical pigment in solution.

(0.00 to 2.00) the ordinates chosen are the logarithms (to base 10) of the numbers 0.01 to 1.00. The scale on the left is chosen to represent the values of the transmittancies. In this way for a given value of T on the left $-\log_{10}T$ can be read at once on the right-hand scale.

The numbers on the right- and left-hand sides of the graph have been plotted so as to make their use convenient, for if one knows the transmittancy (obtained from the instrument) it is only necessary to look on the right-hand side of the chart where the corresponding $-\log_{10}T$ will be found.

Now, if Beer's law [6] holds for dilute carotin solutions (0.00–4.20 mgm. per liter) then transmittancies of different concentrations when plotted on this logarithmic paper should give a straight line, that is, if $bck \equiv -\log_{10}T$, and b and k are kept constant, then c is proportional to $-\log_{10}T$.

[6] Briefly, Beer's law states that the specific transmissive index k is constant regardless of thickness or concentration

An example will serve to illustrate how results are obtained from the spectrophotometric data. Using mercury light of wave length 435.8 and a carotin solution of 0.336 cgm.[7] per liter in a 2 cm. cell, the angles 74.23° and 9.33° were read on the instrument. The angles were designated as θ_1 and θ_2 respectively. The cotangent of θ_1 times the tangent of θ_2 gives the value for the transmittancy, which is 0.0464. The log of 0.0464 is 8.6665 − 10 or − 1.3335 which is given on the right hand side of the graph. These numbers have already been plotted in reference to the transmittancies. Hence, in plotting, or in obtaining the concentration of certain unknown solutions from the lines plotted, it is necessary to know only the value for the transmittancy.

The *specific transmissive index* of carotin for the above wave length is at once found by dividing 1.3335 by 2, the thickness, and by 0.336, the concentration in centigrams. The specific transmissive index will be illustrated by an example later on in this paper.

Data obtained by using the spectrophotometer are usually presented in the form of spectral transmittancy curves. Typical curves are shown in the Bureau of Standards Scientific Paper No. 440 (*3*). The curves of a theoretical pigment have been illustrated in Figure 1. These are drawn for convenience in explaining the method. They represent transmittancies between wave lengths 380 and 670 $m\mu$. Each curve represents a different concentration of the colored solution. The curves differ from each other only by virtue of their concentrations. In order to use such curves for quantitative work it would be necessary to obtain a large number of them, each of which would then represent a definite concentration of pigment solution. Such a series of curves would serve as a standard, with which the curve for an unknown concentration of the same pigment could be compared. This method would be slow and very laborious, for the standard curves would have to be prepared and then several points would need to be determined on the curve of the unknown.

For these reasons a better method was sought whereby an accurate determination could be made by reading only one point on the spectrophotometer.

From each of the three curves at any given wave length (440 for instance) let us obtain the numerical values for the transmittancies. For this wave length they are approximately 0.308 for the 5 cgm. concentration curve, 0.10 for the 10 cgm. and 0.01 for the 20 cgm. curve.

These transmittancy values are, however, plotted in another manner in this chart. At the top of the page, on the X-axis, concentrations (5, 10, 20, cgm. per liter) are represented; at the left-hand side Y-axis of the chart, transmittancies are represented. Now, plotting the transmittancies 0.308, 0.10 and 0.01 at their respective concentrations and drawing a line through these three points and through 1.00 transmittancy, which represents complete transmittancy at zero concentration, the line will be found to be a straight line (marked 440 in fig. 1), extending diagonally across the chart. If wave length 560 is used, then the line marked 560 will be obtained; similarly line marked 380 and 500 for wave lengths 380 and 500; line marked 610 for wave length 610, and so on. Therefore, in the diagram the transmittancies for any wave length which cuts the curve may be used to obtain a straight line, from which quantitative data regarding the concentration of solutions may be obtained.

[7] The carotin used in this test was not pure.

It is readily observable that no interpolations are necessary other than a direct reading from the chart.

Now, if we have transmittancy data for any given wave length and known concentration of a pure dye, to plot accurately a line, such as those above, then, by finding the transmittancy of any unknown concentration of the same dye, the amount of pigment present in the unknown concentration may be readily obtained from the graph. Hence, a rapid spectrophotometric method is obtained for the accurate determination of the concentration of any dye in solution which obeys Beer's law; the manner of using this method for the accurate determination of the amount of carotin in solution will be given in this paper.

THE SPECTRAL TRANSMITTANCY OF CAROTIN, USING HELIUM AND MERCURY LIGHT; THE QUANTITATIVE DETERMINATION OF CAROTIN

COMPARISON OF VALUES FOR THE DIFFERENT WAVE LENGTHS; SELECTION OF MOST SUITABLE WAVE LENGTH; SELECTION OF THE MOST SUITABLE CONCENTRATION OF CAROTIN

Both white light and monochromatic light were available for use with the spectrophotometer. White light was obtained by reflecting the light of ten 600-watt gas-filled tungsten lamps from surfaces composed of magnesium oxide or carbonate (*3*). Helium or mercury lamps were the sources of the monochromatic light used.

The strongest absorption of carotin is in the blue region of the spectrum where the brightness of the white light is too low for accurate photometric measurements. If the slit were widened to obtain sufficient field brightness, errors due to the wide slit and the continuous spectrum of the source of light would be introduced.

In using the mercury and the helium lamps the scale of the instrument could be set on any one of the lines in their spectra, and the slit widened to increase the quantity of light admitted to the instrument, without introducing any error due to finite slit width. The brightness thus obtained was ample for precise photometric work at the following wave lengths: Hg 435.8: He 447.2, He 501.6, Hg 546.1, Hg (576.9 + 579.1) and He 587.6.

Transmittancy curves (3) obtained by the use of white light show the amount of transmittancy in the different regions of the spectrum and, consequently, by making a continuous curve of the absorption of any given solution, its concentration can be ascertained from curves drawn for known concentrations. This method for the determination of the carotin content is accurate but, as explained above, is quite tedious, for a great number of determinations would need to be made at different wave lengths. Now, in seeking a means by which only one determination on the spectrophotometer need be made so that the pigment content of any solution of carotin could be quickly and accurately determined, the complete transmittancy curve for one definite concentration must be known with a fair degree of accuracy.[8]

From wave length 500–720 the transmittancy of carotin is very high for all concentrations; the transmittancy curve for carotin shows bands below 500, that is, it is a region where the value of the transmittancy of carotin in solution changes rapidly, hence any line below 500, within the

[8] The spectral transmission properties of carotin have been worked out by the Colorimetry Section of the Bureau of Standards and are to to be published soon.

limits of practicability, will give accurate values of transmittancy, which values may be readily used in quantitative work. However, the concentration of this solution or thickness of the layer must be such as to bring the value of transmittancy within the range of the instrument for accurate measurement.

The bright helium lines 447.2 and 501.6 and the bright mercury lines, yellow (576.9 + 579.1), green 546.1 and blue 435.8 were used in the preliminary work on carotin with the König-Martens instrument. Transmittancies were then determined for several dilutions of an ethereal carotin solution for each of these wave lengths of light.[9]

A scale of concentrations was chosen such that the values of $-\log_{10} T$ obtained for these concentrations at a given thickness and wave length, will fall within the range of accurate measurement. If the concentrations are plotted on the X-axis and $-\log_{10} T$ on the Y-axis, a line connecting the points will extend diagonally across the chart. Then, from the measured transmittancy of an unkown solution, the concentration may be accurately determined from the graph. If the straight line runs almost parallel with the Y-axis then the range of the concentrations of the solutions relative to the range of the instrument would be too small, while if the line runs parallel with the X-axis then the difference in the transmittancy of the solutions would be too small for accurate work. The transmittancies of carotin solutions at wave lengths (576.9 + 579.1), 546.1 and 435.8 for mercury vapor light are recorded in Table I.

TABLE I.—*Preliminary experiments on the transmittancy of solutions of carotin in U. S. P. ether*[a]

Wave length and light source.	Transmittancy.[b]	Carotin per liter.
		Mgm.
Hg. (576.9+579.1)	0.929	42.00
	0.906	37.80
	0.910	33.60
	0.969	25.20
	0.940	16.80
	0.990	8.40
	0.999	4.20
	1.000	2.10
	0.996	1.05
Hg. (546.1)	0.801	42.00
	0.843	37.80
	0.805	33.60
	0.892	25.20
	0.892	16.80
	0.948	8.40
	0.958	4.20
	1.000	2.10
	0.989	1.05
Hg. (435.8)	0.032	4.20
	0.172	2.10
	0.365	1.05

[a] U. S. P. ether contains not less than 95.5 per cent nor more than 97.5 per cent of $(C_2H_5)_2O$, the remainder consisting of alcohol containing a little water.

[b] No attempt should be made to correlate data given in this table with data which follow, for they were used simply to ascertain which wave length would be most suitable for use. The carotin used here was not pure, nor was purity a requisite for the purpose for which the data were used.

[9] The writer is greatly indebted to Irwin G. Priest, H. J. McNicholas and Dr. M. Katherine Frehafer of the Colorimetry Section of the Bureau of Standards for valuable assistance and many helpful suggestions regarding the work.

The mercury line (576.9 + 579.1) was found to be unsatisfactory for solutions of a concentration less than 42.0 mgm. per liter, for the transmittancies obtained were not of sufficient accuracy and gave values which did not show great enough differences to insure accuracy in the determinations, in other words, the graph line would be too nearly parallel with the X-axis.

Readings for the mercury line (576.9 + 579.1) give transmittancies which are of no use for quantitative work, while those for the line 546.1 offer greater promise. In the case of the mercury lines (576.9 + 579.1) (see Table I) practically all of the light was transmitted by the concentrations used (0.0 to 42.0 mgm. per liter), while line 546.1 was transmitted to too great an extent for accurate quantitative work; concentrations as great as or greater than 8.4 mgm. per liter transmitted none of the mercury light at wave length 435.8.

Since the results obtained from using the mercury line 435.8 appeared very favorable (Table I), another experiment was made with solutions which contained less than 8.4 mgm. per liter, the results of which are given in Table II. Concentrations of 8.4 mgm. per liter and above could not be used because of total extinction at the scale setting of 435.8. Transmittancies were also obtained for the helium lines 447.2 and 501.6.

Since, from Table I, it was observed that the concentrations used were too great for light in the blue regions and also because of the fact that the light from the helium tubes is not as bright as that of mercury vapor, lower concentrations were chosen for the work.

TABLE II.—*Preliminary experiments on the transmittancy*[a] *of a solution of carotin (in U. S. P. ether)*

Wave length and light source.	Transmittancy.	Carotin per liter.
		Mgm.
Hg. 435.8	0.0034	7.560
	0.0156	5.040
	0.1320	2.520
	0.3650	1.260
	0.5920	0.630
	0.7040	0.315
He. 447.2	0.0063	5.040
	0.0871	2.520
	0.2850	1.260
	0.5110	0.630
	0.6220	0.315
He. 501.6	0.0756	12.600
	0.1200	10.080
	0.2100	7.560
	0.3530	5.040
	0.5900	2.520
	0.7740	1.260
	0.8870	0.630
	0.8610	0.315

[a] These data should not be correlated with data in other tables, as the figures here were used only in the search for a suitable wave length and a suitable concentration of the pigment. The concentration 0.315 mgm. as seen in fig. 2 gave low transmittancy values for all three wave lengths of light; this fact shows that the error was due to dilution and not to reading the instrument.

The results obtained for the helium lines as well as for the mercury line, reported in Table II, and the graphs of the values of $-\log^{10}$ transmittancy when plotted against concentration, are illustrated in Figure 2. The transmittancies for the mercury line (435.8) and the helium line

(447.2) when plotted give a series of points which can be used very readily in the quantitative determination of carotin. In Figure 2 the concentration of the solution is represented on the X-axis and the $-\log^{10}$ transmittancy on the Y-axis. It was found that readings could be more

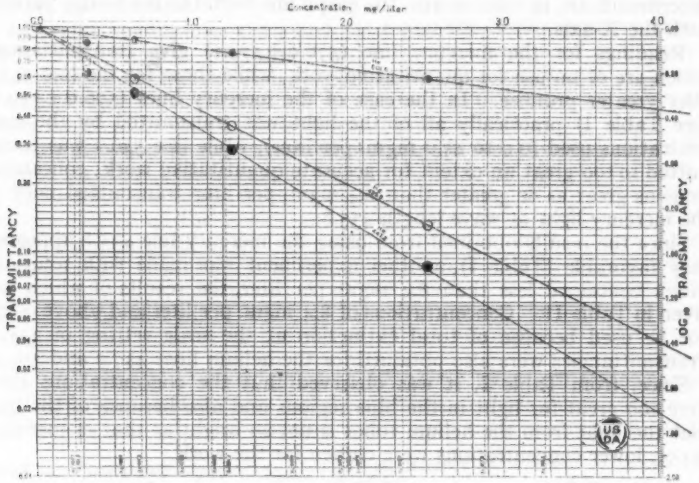

FIG. 2.—Comparison of graphs obtained by using the helium lines 447.2 and 501.6 and the mercury line 435.8.

easily made using the mercury line, because of its greater brightness, but as far as transmittancies and concentrations are concerned the light from either mercury or helium is suitable. Light from the mercury line was used in most of the work, as this source of light is in more common usage and would be more readily available to anyone wishing to do similar work on the quantitative determination of carotin.

COMPARISON OF THE ACCURACY OF READINGS OF THE MERCURY LINE 435.8 AND HELIUM LINE 447.2

It will not be out of place here to compare the results of determinations made by using the mercury line (435.8) and the helium line (447.2) on the same solutions of carotin in a 2 cm. cell.

TABLE III.—*Comparison*[a] *of quantitative determinations made, using helium and mercury light*

Scale.	Transmittancy.	Dilution.	Carotin determined per liter, using graphs in figure 2.
			Mgm.
Hg. 435.8 } Soln. A	0.0799	20 X	63.0
He. 447.2 }	0.0460	20 X	62.6
Hg. 435.8 } Soln. B	0.1492	200 X	474.0
He. 447.2 }	0.0970	200 X	474.0
Hg. 435.8 } Soln. C	0.1175	500 X	1,335.0
He. 447.2 }	0.0654	500 X	1,380.0

[a] The data of this table should not be correlated with those of other tables.

Quantitative determinations were made on three solutions (A, B, and C) of different concentrations. These are recorded in Table III. The results obtained are concordant indicating that either the Hg. or He. line may be used.

THE EFFECT OF SOLVENTS UPON TRANSMITTANCY; TRANSMITTANCY OF A SOLUTION OF PURE CAROTIN

Often, it is convenient to have the carotin dissolved in petroleum ether or alcohol instead of ether and, consequently, some knowledge of the influence of these three solvents upon the transmittancy seemed desirable. The solutions were made as follows: There were dissolved 21.0 mgm. of carefully purified carotin in 250 cc. of petroleum ether.

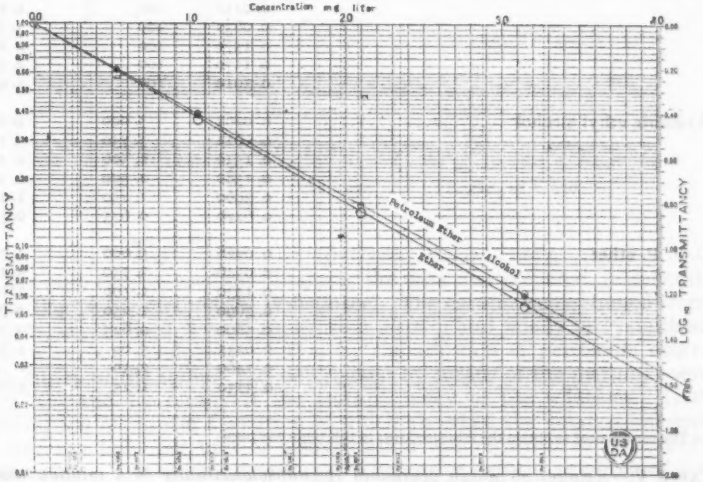

FIG. 3.—Comparison of graphs of the transmittancies of identical concentrations of carotin in petroleum ether, alcohol and ether, using the mercury line 435.8.

Of this solution 2.5 cc. were then diluted to 50 cc. with petroleum ether, ether, or alcohol, and all further dilutions were made with the same respective solvents.[10] It will be noted that the ether and alcohol solutions contain small percentages of petroleum ether. The results are given in Table IV and are plotted in Figure 3.

In Figure 3 the effect of the solvents is seen. The ethereal solution of carotin transmitted somewhat less light than did the alcohol or the petroleum ether solution. The graphs for the petroleum ether and the alcohol solutions coincide. Since the results (Table IV) for ether were obtained from two different weighings and preparations of carotin and checked within the limits of error, they have been used as the basis for quantitative determinations.

To check the accuracy of the graph for the carotin sample given in Figure 3, 42.0 mgm. of a third specially purified sample of carotin were

[10] The petroleum ether used here was the fraction which boils at 30-50° C., the ether was U. S. P. and the alcohol absolute.

dissolved in 250 cc. of petroleum ether. Two cc. of this solution of carotin were diluted to 100 cc. with U. S. P. ether and the transmittancy determined. The transmittancy is given in Table V and is determined from the graph; 41.9 mgm. of carotin were found in the solution, which contained 42.0 mgm. by weight.

TABLE IV.—*Comparison of the transmittancies of identical concentrations of carotin in petroleum ether, in alcohol, and in ether, using the mercury line 435.8*

Solvent.	Transmittancy.	Percentage of petroleum ether in the solution.	Carotin per liter.[a]
			Mgm.
Petroleum ether	0.0246	100	4.20
	0.0252	100	4.20
	0.0632	100	3.15
	0.1570	100	2.10
	0.1570	100	2.10
	0.3910	100	1.05
Absolute ethyl alcohol	0.0263	5.000	4.200
	0.0612	3.750	3.150
	0.1530	2.500	2.100
	0.1580	2.500	2.100
	0.3960	1.250	1.050
	0.6220	0.625	0.525
U. S. P. ether	0.0228	5.000	4.200
	0.0546	3.750	3.150
	0.1430	2.500	2.100
	0.3690	1.250	1.050
	0.5890	0.625	0.525
	0.0235	5.00	4.20
	0.1460	2.50	2.10
	0.3730	1.25	1.05

[a] The carotin used to obtain the measurements in this table was pure.

TABLE V.—*Amount of carotin determined spectrophotometrically in a solution which contained 42.0 mgm. per liter by weight*

Scale.	Transmittancy.	Dilution.	Milligrams of carotin found per liter.
435.8	0.0464	12.5 x	41.9

The specific transmissive index for carotin in petroleum ether, alcohol or ether can then be calculated from the data obtained from the graphs in Figure 3, using the equation—

$$k \equiv \frac{-\log_{10} T}{bc}.$$

The average value for k, the specific transmissive index (extinction coefficient) for carotin in petroleum ether or alcohol for the mercury line 435.8 is found to be 1.915, while that for ether is found to be 1.990.

TABLE VI.—*Specific transmissive index for carotin*

Concentration (C).[a]	$-\log_{10}T$	Thickness (b).	Constant (k).
IN PETROLEUM ETHER OR IN 95 PER CENT ALCOHOL.			
0.400	1.530	2 cm.....	1.912
0.370	1.418	2 cm.....	1.916
0.290	1.110	2 cm.....	1.913
0.130	0.499	2 cm.....	1.919
0.070	0.268	2 cm.....	1.914
		Average....	1.9148, or 1.915
IN ETHER.			
0.400	1.585	2 cm.....	1.981
0.370	1.467	2 cm.....	1.982
0.290	1.150	2 cm.....	1.982
0.130	0.519	2 cm.....	1.996
0.070	0.281	2 cm.....	2.007
		Average....	1.9896, or 1.990

[a] Cgm. per liter.

The pure carotin used for obtaining the data was prepared, in general, according to the methods of Willstätter. The carotin was obtained from carrots, crystallized several times from alcohol and finally recrystallized from a carbon disulphide solution by the addition of petroleum ether. The purity of the sample was determined by means of the melting point (174° C.) and by means of the transmittancy of the solutions. That preparation of carotin a solution of which absorbed the greatest amount of mercury light per unit of concentration was regarded as the purest obtainable. The specific transmissive index for carotin as obtained for ether is based upon the results for three different preparations and three different weighings of carotin, after considerable preliminary investigation. The complete details of the preparation of carotin will appear in a paper which will follow.

BRIEF OUTLINE OF THE PROCEDURE FOR THE DETERMINATION OF CAROTIN BY THE SPECTROPHOTOMETRIC METHOD

The carotin is obtained in ethereal or petroleum ether solution in the method [11] of its separation and is ready for spectrophotometrical examination. The carotin solution is made up to some convenient volume, for example, 100 or 500 cc. It will most likely be necessary to make dilutions from this, for the solution used in the spectrophotometer must contain not more than 4 milligrams of carotin per liter, because greater concentrations will give a transmittancy which can not be used in obtaining data from the graphs in Figure 3.

[11] The method will be described in a later paper.

For the experiments in this paper each cell had an inside measurement of 2 cm. The 2 cm. refers to the thickness of the solution through which the light passes and not to the diameter of the cell. One cell is filled with the carotin solution and the other with the solvent. The cells are then placed in the holder on the instrument. In measuring transmittancies, as in this investigation, the cell containing the carotin solution is placed in one beam of light, and a similar cell, containing the solvent, is placed in the other beam. These cells are interchanged and the nicol is rotated in each case until an intensity match is obtained.[13] The transmittancy

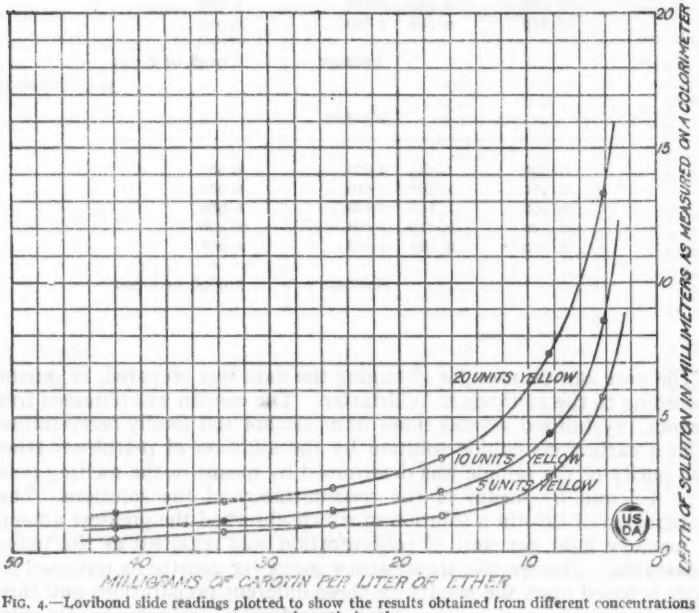

FIG. 4.—Lovibond slide readings plotted to show the results obtained from different concentrations of carotin in ether.

is then obtained as the product of the cotangent of the angle in one case by the tangent of the angle in the other.

With an ethereal solution of carotin, using the mercury line 435.8, angles were read as follows: θ_1, 64.18; θ_2, 16.43. The cotangent of θ_1 times the tangent of θ_2 gives the value 0.143 for the transmittancy of the solution. At the left-hand side of Figure 3, the value 0.143 for the transmittancy is found. This point on the graph represents 2.12 mgm. of carotin per liter. The transmittancy, and consequently the concentration, of any solution of carotin of unknown concentration may be obtained in this way.

[13] For each position of the cells there are four settings of the nicol for which an intensity match can be obtained; that is, one in each quadrant of the circle. The instrument was adjusted so that the readings could all be taken in the first quadrant; usually 10 settings of each angle of match were made. In using the homogeneous light source, such as was used in these experiments, the ocular and the collimator slits were opened to 0.5 mm. and the field brightness thus obtained was ample in all cases to make good settings of the angle of match.

THE COLORIMETRIC METHOD OF DETERMINING CAROTIN

Besides determining the amount of carotin in a solution spectrophotometrically it has been determined colorimetrically in the present work. Considerable work was done on the carotin content of many solutions, and it was for this purpose that the method which follows was devised. The method is based upon certain standard Lovibond slides, 5, 10, and 20 yellow, used in connection with known solutions of carotin, which were matched in a Duboscq colorimeter. The Lovibond slide was placed on the left side on the stand which holds the cell (the cell remaining in position) and the solution of known concentration was placed in the cell on the right side. Care was taken that the right side gave exactly

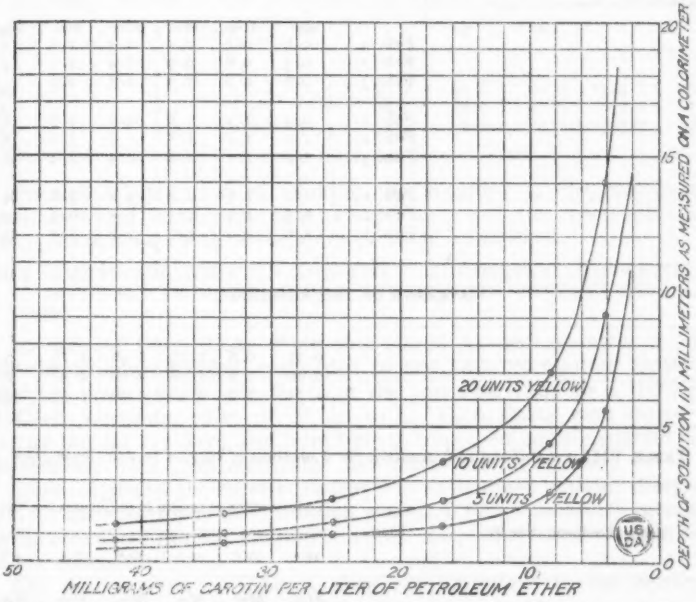

FIG. 5.—Lovibond slide readings plotted to show the results obtained from different concentrations of carotin in petroleum ether.

a zero reading. The right cell was then adjusted so that the column of the liquid (carotin in petroleum ether, ether, or alcohol) matched the Lovibond slide. Curves were drawn from the readings obtained; the concentration in grams per liter is represented on the X-axis, while the depth in millimeters is plotted on the Y-axis. Each of the reported readings is an average of from 3–10 settings on the colorimeter. The results are averaged in the lower part of Tables VII and VIII, and from these the curves in Figures 4 and 5 are drawn.

It was intended at first to use these curves for the accurate determination of carotin in solutions, but the more accurate method with the spectrophotometer was later developed. The results of a reading on a solution of carotin in ether of unknown concentration by way of illustration will be cited here as an example. An average of 10 settings for each Lovibond slide gave 1.1 mm. for the 5 slide, 1.6 for the 10 slide and

2.7 for the 20 slide. By looking at Figure 4 it is found that 1.1 mm., on the curve for the 5 slide, represents 23.4 mgm. per liter; 1.6, on the curve for the 10 slide, represents 24.8 mgm. per liter; and 2.7, on the curve for the 20 slide, represents 22.0 mgm. per liter; or, when averaged, they show that the solution under observation contained 23.4 mgm. of carotin per liter.

TABLE VII.—*Lovibond slide readings for a solution of carotin in ether*

Yellow Lovibond slide No.	Date.	Concentration of carotin in milligrams per liter.					
		42.0	33.6	25.2	16.8	8.4	4.2
		Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
5	Feb. 1	0.6	0.8	1.1	1.3	2.7	6.2
	Feb. 2	0.5	0.8	0.9	1.4	2.7	4.9
	Feb. 3	0.6	0.8	1.1	1.5	2.9	5.9
10	Feb. 1	0.9	1.2	1.6	2.3	4.5	8.1
	Feb. 2	1.0	1.2	1.5	2.2	5.2	8.6
	Feb. 3	1.0	1.2	1.6	2.3	4.4	8.8
20	Feb. 1	1.6	1.8	2.2	3.2	7.8	14.0
	Feb. 2	1.5	1.9	2.5	3.4	6.9	12.3
	Feb. 3	1.5	2.0	2.7	3.8	7.3	13.6
AVERAGE OF THE READINGS							
5		0.57	0.8	1.03	1.40	2.76	5.7
10		0.96	1.2	1.56	2.26	4.36	8.5
20		1.53	1.9	2.46	3.46	7.33	13.3

TABLE VIII.—*Lovibond slide readings for a solution of carotin in petroleum ether*

Yellow Lovibond slide No.	Date.	Concentration of carotin in milligrams per liter.					
		42.0	33.6	25.2	16.8	8.4	4.2
		Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
5	Feb. 1	0.5	0.7	1.1	1.2	2.3	6.0
	Feb. 2	0.5	0.7	1.0	1.4	2.5	4.9
	Feb. 3	0.5	0.7	.....	1.5	2.9	5.1
10	Feb. 1	0.7	1.0	1.5	2.2	4.2	9.7
	Feb. 2	1.0	1.1	1.5	2.3	4.5	8.7
	Feb. 3	0.8	1.1	.....	2.4	4.5	9.0
20	Feb. 1	1.2	1.5	2.1	3.2	6.7	15.5
	Feb. 2	1.6	1.8	2.6	3.8	7.0	13.0
	Feb. 3	1.4	1.9	.....	4.2	7.3	13.5
AVERAGE OF THE READINGS							
5		0.50	0.70	1.05	1.36	2.56	5.33
10		0.83	1.06	1.50	2.30	4.40	9.13
20		1.40	1.73	2.35	3.73	7.00	14.00

It was of great interest to know just what might be expected of such colorimeter readings, so checks on the method were attempted. In one case an unknown solution of carotin in ether was taken and readings made on the solution; this solution was then diluted to 50 per cent of its original strength, and again to 25 per cent; readings were then made on the three different concentrations of the carotin solutions.

TABLE IX.—*Lovibond slide readings obtained to determine the amount of carotin in the solution*

Concentration (per cent).	Lovibond slide reading.			Respective carotin content as determined by each Lovibond slide.			Carotin content per liter average.
	5	10	20	5	10	20	
100	1.1	1.6	2.7	23.4	24.8	22.0	23.4 X 1 23.4
50	2.2	3.5	5.2	10.0	10.4	11.6	10.7 X 2 21.4
25	3.8	6.9	11.5	5.4	5.2	5.0	5.5 X 4 22.0

Perhaps a better way of securing the accuracy of the colorimeter readings would be to make direct comparisons with the spectrophotometer. In this way both of the methods could be checked.

THE SPECTROPHOTOMETER AND THE COLORIMETER COMPARED AS TO ACCURACY IN DETERMINING THE CAROTIN CONTENT OF SOLUTIONS

In each of three flasks there was placed a small quantity of pure carotin; absolute alcohol was added to one flask, to another Squibb's absolute ether (taken directly from the container) and to a third redistilled petroleum ether (B. P. 30–50°). These flasks were stored in the ice box for 143 days, and from time to time their carotin content was determined both colorimetrically and spectrophotometrically.

Colorimetrically the carotin content was determined without dilution; but for the spectrophometer dilution was often found necessary because, in the case of carotin, the instrument is best adapted for use with solutions of low concentration.

The results given in Table X show the variations in the readings obtained with the colorimeter and the spectrophotometer.

TABLE X.—*A comparison of results obtained on the same solution of carotin by means of the colorimeter and the spectrophotometer*

Age of solution in days.	Absolute alcohol.		Absolute ether.		Petroleum ether.	
	S.[a]	C.[b]	S.	C.	S.	C.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
0	3.64	.....	43.60	.....	50.80	.....
24	3.58	3.9	10.12	8.7	.....	45.0
38	3.68	3.8	6.32	3.8	49.00	47.0
50	3.63	3.2	3.24	.....	49.00	41.0
71	3.60	3.3	2.05	.....	50.40	43.7
111	3.55	.....	1.16	.....	50.00	.....
143	3.54	.....	0.83	.....	52.40	.....

[a] S=Spectrophotometric results. [b] C=Colorimetric results.

The results for the alcoholic solutions are the most comparable because for both instruments the solution was not diluted before observations were made, and, further, alcohol is less volatile, hence any errors from this source would be reduced. The alcoholic solution was more advantageous for the colorimeter, for experimenters generally agree that readings made from dilute solutions are more accurate than those from more concentrated ones. It will be observed that for alcohol some of the results for the colorimeter are greater and some are less than those for the spectrophotometer.

In the case of ether, the pigment oxidized and, consequently, only two results were obtained by means of the colorimeter. The other points could not be gotten, for the concentration of the pigment became too small to be read from the graph in Figure 4.

By diluting the concentration used it was always possible to obtain readings by means of the spectrophotometer. In this respect the spectrophotometer was superior to the colorimeter, for it was always possible to make dilutions on the solutions used.

The petroleum ether solution of carotin was diluted 20 times before making the determinations on the spectrophotometer, while it was not diluted at all for the determinations on the colorimeter.

In every way the best comparison is in the case of absolute alcohol. The results show very clearly what may be expected of the two instruments, under the conditions given. The spectrophotometer gave results which were quite consistent, while the colorimeter gave results which varied.

In Table X it is seen that the carotin content as determined in the alcoholic solution by means of the spectrophotometer varied between 3.54 and 3.68 mgm. per liter, with an average of 3.60, which is a maximum deviation of +.08 and −.06, or a maximum determination error of 14 parts in 360, or 3.9 per cent. In the case of petroleum ether the results varied between 49.0 and 52.4 mgm. per liter, with an average of 50.3, which is a maximum deviation of +2.1 and −1.3, or a maximum determination error of 34 parts in 503, or 6.7 per cent. It must be remembered that the petroleum ether solution was diluted 20 times, while the alcoholic solution was not diluted, so that the results as actually measured on the spectrophotometer varied between 2.45 and 2.62 mgm. per liter in the case of the petroleum ether solution.

The figures (Table X) which were obtained for the alcoholic solution by using the colorimeter vary from 3.2 to 3.9 with an average of 3.55, a maximum deviation of +0.25 and −0.35, or a maximum determination error of 60 parts in 355, or 16.9 per cent.

Escher (*2*, *p.* 47) is the only investigator who gives the individual colorimeter readings. His readings are 82.0, 82,0, 83.8, 84.4, 83.6, 87.5, 87.9, 87.5, giving an average of 84.8 divisions on the colorimeter. His maximum determination error was 5.9, or 6.9 per cent. In other sets of readings his maximum errors were 8.4, 4.2, and 5.1 per cent.

Some figures obtained by three different observers, all of whom were more or less familiar with the colorimeter, are submitted herein. A carotin solution (1.68 parts in 100,000 of ether) was used, and the same solution was put into both cells of the Duboscq colorimeter. The left-hand side was set at 50 mm. and each of the three observers, A, B, and C, regulated the right-hand side so as to match the solution on the left, while the writer observed the readings. Observers A and B by shading their eyes with a cloth got the results in AA and BB just a few minutes

after the first readings were made. All agreed that matching the two solutions was very difficult.

TABLE XI.—*Readings made by different observers on the same solution, showing the inaccuracies of the colorimeter method as generally used*

A.	B.	C.	AA.	BB.
47.5	46.8	47.0	58.0	48.4
40.6	43.8	46.8	59.8	49.8
42.5	44.3	45.4	60.7	52.0
46.2	44.6	46.6	50.3	48.2
43.6	49.4	45.8	60.5	49.4
44.1	45.8	46.3	59.9	49.6

When readings such as the above are obtained (Table XI) and in view of the unknown errors of other workers, it is readily seen that the work of various investigators on the carotin content of different substances can not be compared as to the absolute amount of carotin present. The results of any one worker should be of some value in giving him comparative figures to be used only in his own work; but one does not see how it is possible to assign actual values to data submitted in the literature and have such values mean more than a rough approximation of the amount of carotin actually present.

SUMMARY

The spectrophotometric method for the determination of carotin is described and graphs are given for determining the amount of carotin in solutions when the transmittancy is known.

The mercury line 435.8 was finally adopted as the best scale on the spectrophotometer for giving the most accurate results with carotin solutions.

Solutions of the same concentration of carotin in alcohol and petroleum ether gave approximately the same transmittancies, while the transmittancy for ether was a little less.

The specific transmissive index (extinction coefficient) of carotin in alcohol and petroleum ether for the mercury line 435.8 was found to be 1.91; in ether 1.986.

Quantitative determinations of carotin by means of the colorimeter and the spectrophotometer were compared; the colorimeter was found to be unsatisfactory because of the difficulty in matching the solutions, thus giving a greater percentage of error, while the spectrophotometer gave excellent results both as to accuracy and ease of reading the instrument.

In working with the spectrophotometer it is not necessary to prepare pure carotin as a standard or use any other standard, for the instrument itself is standardized in regard to carotin when the transmittancy of its solution is known.

The transmittancies as measured depend upon the physical properties of the substances involved, and not upon variability in light, physiological factors, or tint of solution, which so greatly affect the readings in any colorimeter, and are independent of abnormalities of the observer's color vision.

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LITERATURE CITED

(1) ARNAUD, A.
1887. DOSAGE DE LA CAROTINE CONTENUE DANS LES FEUILLES DES VÉGÉTAUX. *In* Compt. Rend. Acad. Sci. [Paris], t. 104, p. 1293–1296.

(2) ESCHER, Heinrich H.
1909. ZUR KENNTNIS DES CAROTINS UND DES LYCOPINS. 102 p., 1 col. pl. Zürich. Bibliographical footnotes.

(3) GIBSON, K. S., and others.
1922. THE SPECTRAL TRANSMISSIVE PROPERTIES OF DYES: I. SEVEN PERMITTED FOOD DYES, IN THE VISIBLE, ULTRA-VIOLET, AND NEAR INFRA-RED. U. S. Dept. Com., Bur. Standards Sci. Paper 440, p. 121–184, 64 fig.

(4) ——— and MCNICHOLAS, H. J.
1919. THE ULTRA-VIOLET AND VISIBLE TRANSMISSION OF EYE-PROTECTIVE GLASSES. U. S. Dept. Com., Bur. Standards Tech. Paper 119, 47 p., 25 fig.

(5) JACOBSON, C. A.
1912. ON THE COLORING MATTERS IN ALFALFA, ALFALFA INVESTIGATION, III. *In* Jour. Amer. Chem. Soc., v. 34, p. 1263–1266. Bibliographical footnotes.

(6) MARTENS, F. F., and GRÜNBAUM, F.
1903. ÜBER EINE NEUKONSTRUKTION DES KÖNIGSCHEN SPEKTRALPHOTOMETERS. *In* Ann. Phys., Folge 4, Bd. 12, p. 984–1003, 7 fig. Bibliographical footnotes.

(7) MATHEWSON, WALTER E.
1916. THE USE OF THE SPECTROPHOTOMETER FOR THE EXAMINATION OF FOOD COLORING MATTER. *In* Jour. Assoc. Off. Agr. Chem., v. 2, p. 164–169.

(8) ———
1920. THE COMBINATION OF FRACTIONATION WITH SPECTROPHOTOMETRY IN PROXIMATE ORGANIC ANALYSIS. *In* Jour. Amer. Chem. Soc., v. 42, p. 1277–1279.

(9) ———
1920. THE DETECTION AND ESTIMATION OF YELLOW AB AND YELLOW OB IN MIXTURES. *In* Jour. Indus. and Engin. Chem., v. 12, p. 883–887. Bibliographical footnotes.

(10) ———
1921. REPORT ON COLORING MATTERS IN FOODS. *In* Jour. Assoc. Off. Agr. Chem., v. 5, p. 196–218, 2 fig.

(11) MONTEVERDE, N. A., and LUBIMENKO, V. N.
1913. IZELI͡EDOVANIIA NAD OBRAZOVANIEM KHLOROFILLA U RASTENII͡. III. O PRIMI͡ENENII SPEKTROKOLORI METRICHESKAGO METODA KOLICHESTVENNAGO ANALIZA PRI IZUCHENII VAPROSA O NAKOPLENII KHLOROFILLA, KSANTOFILLA I KAROTINA O RASTENII. FORMATION OF CHLOROPHYLL IN PLANTS. III. APPLICATION OF THE SPECTRO-COLORIMETRIC METHOD OF QUANTITATIVE ANALYSIS TO THE STUDY OF THE ACCUMULATION OF CHLOROPHYLL, XANTHOPHYLL AND CAROTIN IN PLANTS. *In* Bul. Acad. Imp. Sci. St. Pétersb. sér. 6, t. 7, p. 1007–1028, illus. Bibliographical footnotes.

(12) PALMER, LEROY S.
1922. CAROTINOIDS AND RELATED PIGMENTS. 316 p., 1 fig., 2 pl. New York. Bibliography, p. 279–295.

(13) WURMSER, RENÉ, and DUCLAUX, J.
1920. SUR LA PHOTOSYNTHÈSE CHEZ LES ALGUES FLORIDÈES. *In* Compt. Rend. Acad. Sci. [Paris], t. 171, p. 1231–1233.

OUR ONLY COMMON NORTH AMERICAN CHIGGER, ITS DISTRIBUTION AND NOMENCLATURE[1]

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Since beginning the study of our North American chiggers a few years ago the writer has collected many specimens in different parts of the United States, and in addition has received many more specimens from various entomologists. It is believed that these collections have been sufficiently numerous and diversified geographically to give a good index to the occurrence and distribution of our chiggers.

The outstanding fact that has been established by the study of these chiggers is that in nearly all localities the common species attacking man and domestic animals is *Trombicula tlalzahuatl* (Murray). This species is now known to occur from Long Island to central Mexico and from the Atlantic coast to the Rocky Mountains. It has been found on lands actually flooded at times by marine tide waters and also occurs well up in the Appalachian Mountains. In the more humid southern part of its range the mite is found almost everywhere where there is rough growth of woods or shrubbery. Toward the northern limits of its range the species occurs only in isolated "islands" where the local conditions are favorable for its maintenance.

This common species of the United States, so long known yet so little studied, appears to be no other than the well-known Mexican chigger with the unpronounceable specific name which is spelled two ways, either *tlalzahuatl* or *tlalsahuate*. This species should be referred to the genus Trombicula, not to Microthrombidium, as has been done by different workers in the past. For a long time the writer had been of the belief that our chigger of the Atlantic seaboard and lower Mississippi Valley was *tlalzahuatl*, but it remained to get further evidence before coming to a definite conclusion. This was obtained recently when an examination was made of many specimens from Texas and other Southern States. Not only is our common chigger species found over apparently the whole of the Gulf States, except of course where local conditions prohibit, but it increases in its abundance as the coast is approached; and at points in Texas as far south as Houston the writer has found infestation in maximum proportions. Thus the conclusion is justified that the same species ranges far to the southward, in fact into the moist lowlands of Mexico, where *tlalzahuatl* has long been known.

In 1912 Oudemans [2] gave good drawings and a good description of *tlalzahuatl*, types of which are now in the Trouessart collection. Until this description appeared it would have been impossible to recognize the Mexican chigger without actually seeing specimens of it. By comparing specimens of our common chigger with Oudeman's figures and description

[1] Accepted for publication August 11, 1923.

[2] OUDEMANS, A. C. 1912. DIE BIS JETZT BEKANNTEN LARVEN VON THROMBIDIIDAE UND ERYTHRAEIDAE. *In* Zool. Jahrb., Sup. 14, p. 18-24, fig. D.

it becomes apparent that there is agreement in all the essential characters; thus the writer feels confident in pronouncing our common species *tlalzahuatl*.

As to the common name of *tlalzahuatl:* In view of the fact that the name Mexican chigger has not been very generally used and is now, according to our most recent knowledge of the distribution of this species, inappropriate, the writer is taking the liberty of suggesting here a new common name for this species. That of the common North American chigger would be distinctive, or the species could be called just the North American chigger, as it far exceeds all others in numbers and importance and is the only one attacking man and domestic animals that is distributed over a large portion of the continent.

Recently Hirst [3] has figured under the name of *Leptus* (*Trombicula*?) *similis* a chigger taken from chickens in the United States which is identical with the common one infesting chickens and attacking man and apparently all of our domestic animals—i. e., *Trombicula tlalzahuatl* (Murray).

It is probable that the chigger described by C. V. Riley [4] as *Leptus irritans* is no other than our common species. Until a further study is made of the exceedingly poor specimen of *irritans* left in the old Riley collection, this point can not be settled definitely. About all that remains of this injured Riley specimen is a jaw. Knowledge of the structure of the chelicerae alone has been insufficient in the past for specific determination but may soon prove sufficient as our knowledge increases.

The adult form of our common chigger is very probably *Trombicula cinnabaris* Ewing,[5] the only *Trombicula* adult known to be widely distributed in the region infested by *Trombicula tlalzahuatl* (Murray).

Specimens of *Trombicula tlalzahuatl* (Murray) [6] have been determined by the writer from the following localities and hosts:

New Jersey:
Pemberton, one specimen from man, July 19, 1915 (H. K. P.).
Pennsylvania:
Stone Valley, Huntingdon County, many specimens, August 3, 1922 (D. L. Van Dine).
Maryland:
Berwyn, four specimens from man, July, 1919 (A. B. Gahan).
Bannockburn golf grounds, near Glen Echo, one specimen attached to man, summer of 1919 (H. E. Ewing).
Chesapeake Bay, hundreds of specimens attached to king snake, summer of 1920 (W. Palmer).
Somerset, several specimens attached to man, August 16, 1919 (H. E. Ewing).
North Beach, one specimen attached to man, September 21, 1919 (H. E. Ewing); many specimens in leaves and trash, also on man, summer of 1921 (H. E. Ewing); many specimens on and attached to man, summer of 1922 (H. E. Ewing).
Takoma Park, several specimens attached to man, summers of 1921 and 1922 (H. E. Ewing).
Virginia:
East Falls Church, a few engorged specimens in scrapings from concealment nest of rabbit, August 21, 1919 (H. E. Ewing); many specimens in trash on surface of ground and attached to man, summer of 1919.
Great Falls, two specimens attached to man, August 18, 1919, and one specimen attached to man, August 24, 1919 (H. E. Ewing).

[3] HIRST, Stanley. 1922. MITES INJURIOUS TO DOMESTIC ANIMALS. Brit. Mus. Nat. Hist. Econ. Ser. No. 13, p. 78.
[4] RILEY, C. V. 1873. HARVEST MITES. *In* Amer. Nat., v. 7, p. 17-18, fig. 3b.
[5] EWING, H. E. 1920. THE GENUS TROMBICULA BERLESE IN AMERICA AND THE ORIENT. *In* Ann. Ent. Soc. Amer., v. 13, p. 387-389, fig. 3.
[6] MURRAY, Andrew. 1877. ECONOMIC ENTOMOLOGY. APTERA. p. 113. London.

Florida:
Orlando, many specimens attached to man, June, 1922 (H. E. Ewing).
Royal Palm State Park, one specimen attached to man, June 6, 1922 (H. E. Ewing).
Louisiana:
Tallulah, several specimens attached to man, October, 1921 (H. E. Ewing).
Mound, several specimens attached to man, October, 1921 (H. E. Ewing).
Texas:
Houston, several specimens attached to man, October 19, 1921 (H. E. Ewing).
Duncanville, several specimens on jack rabbit, September 15, 1920 (Bishopp No. 9183); specimens from rabbit, September 15, 1920 (Bishopp No. 9187); many specimens on cottontail rabbit, September 24, 1921 (Bishopp No. 10093).
Missouri(?):
One specimen with chelicerae attached to skin of some animal, May 28, 1880 (H. Lugger (?)).
Iowa:
Keosauqua, several specimens attached to man, June 25, 1920 (H. E. Ewing).
Kansas:
Highland, several specimens on man, July, 1905 (P. A. Glenn).

Specimens with host records only are as follows: Squirrel, one specimen; Citellus, two specimens; prairie dog, several specimens; cat, two specimens; chicken, two lots of specimens; snake, one specimen; rat, two specimens.

HABITS OF THE COTTON ROOTROT FUNGUS[1]

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INTRODUCTION

In recent years much study has been given to a disease of cotton and alfalfa commonly known as rootrot, which occurs chiefly in certain sections of Texas, New Mexico, and Arizona. It is generally believed that this disease is caused by a soil-inhabiting fungus which attacks the roots of the host plants and by invading and breaking down the tissues of the roots, cuts off the water supply and causes death. This fungus was described by Shear (*8*) [2] as a facultative parasite with sterile mycelium, and was named by him *Ozonium omnivorum* [3]

While the occurrence of a disease called rootrot had been observed for over 20 years in the Salt and Gila River Valleys of Arizona it was not until recent years, when a large percentage of the cultivated areas in these valleys was planted to cotton, that the disease received much notice or caused much alarm. This probably is owing to the fact that the disease is never so striking to the eye when occurring in alfalfa fields as it is when present in cotton fields. In alfalfa fields the dead areas often are invaded quickly by grasses and weeds of different kinds which, being green in color, conceal the fact that the alfalfa plants have been killed. There is a tendency also for a considerable number of alfalfa plants in the dead areas to recover by sending out new lateral roots from small portions of the old roots which are not killed back completely but remain alive just below the crown. Often there are sufficient numbers of these reestablished plants to prevent the recently infected plants from receiving notice. In cotton fields a striking contrast is noticeable between the black and brown streaks and patches of dead plants and the green areas of healthy plants.

It appears from the literature that practically all of the study that has been given to this disease by different investigators has been made with cotton as the host plant. While some descriptions have mentioned the fact that the disease spreads in a more or less centrifugal manner, others (*4*) have stated that "the dead patches have no definite boundaries, but extend in all directions through the field." While studying the disease in its relation to soil conditions in the Salt and Gila River Valleys during the seasons of 1918 to 1922, inclusive, the writer was struck by the fact that frequently in alfalfa fields almost perfect circles were formed by the dead and dying plants as the disease spread outward in all directions from a central plant which was the first to show injury.

[1] Accepted for publication Aug. 11, 1923.

[2] Reference is made by number (italic) to "Literature cited," p. 418.

[3] Duggar (*1*), after finding in 1915 what he believed to be the conidial stage of the fungus, tentatively assigned the organism to a new genus, *Phymatotrichum*.

DEATH RATE OF ADJACENT PLANTS

In order to determine if the rate of progress was the same in all directions from the center, the progressive spread was measured in nine circular spots during the summer of 1922. The spots, when selected on June 23, were almost perfect circles, and on that date three of them were approximately 5 meters in diameter, three others were about 3 meters in diameter, and the other three were 1½ meters in diameter. About each of these spots two circles were described, 15 and 35 cm.

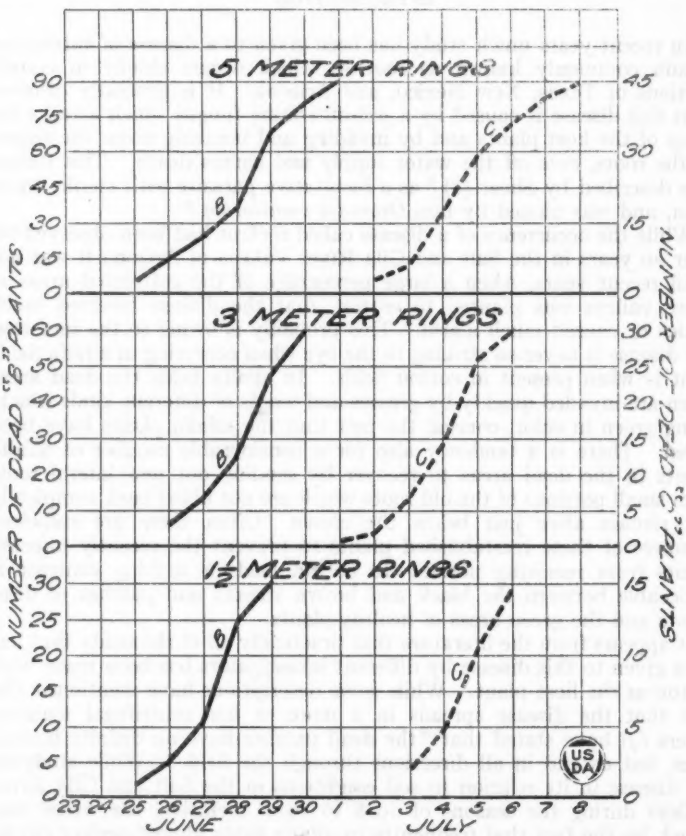


FIG. 1.—Graphical representation of the death rate of alfalfa plants in rootrot circles of different diameters. The solid lines indicate the number of dead "B" plants, and the dotted lines the number of dead "C" plants, or those on the outer circles.

outside the ring of dead plants. On the first of these circles plants were selected and tagged as "B" plants, 90 in the 5-meter spots, 60 in the 3-meter spots, and 30 in the meter and a half spots. In each case the plants were spaced at approximately equal distances. In the outer circle 45, 30, and 15 plants were similarly selected and tagged as "C" plants. Observations were made each day on the 15 days following,

and the date upon which each plant wilted was written upon the tag attached. The progressive death rate of all of the tagged plants in the three sizes of spots is illustrated in Figure 1.

It will be noted that none of the tagged plants in the outer circle (C plants) were attacked by the disease until after the death of all of the tagged plants in the intermediate circle (B plants). Although the plants on these circles did not die simultaneously, the sequence in which the different circles of plants were attacked seems to indicate that the centrifugal manner in which the disease spreads in alfalfa fields is not the result of accident.

CONIDIAL STAGE OF THE FUNGUS

Duggar (*1*) reports having observed in 1902 a circular spot or incrustation of spores in the vicinity of a dead spot of cotton in Texas. He did not associate this with the fungus on roots of the diseased cotton plants until several years later, when he again examined the collected material. In September, 1915, he found an abundance of similar material in a field of diseased cotton near Paris, Tex. He was unable to find any of the conidial areas in the earlier stages of development, but was able to observe the method of spore formation by studying material from the periphery of the circular mass.

During the summer of 1917 the writer observed spore masses corresponding to Duggar's description in 8 of 20 infected cotton fields, three times under dying umbrella trees (*Melia Azedarach umbraculiformis*) and in three of eight infected alfalfa fields. In 1918, after a week of rainy weather in early August, the spore material was found in 10 of 13 cotton fields where the presence of rootrot had been suspected or identified. A few mats of spore material were observed in infected cotton fields in 1919, but none in 1920. During the seasons of 1917 to 1920, inclusive, observations were confined almost entirely to cotton fields. During that time no particular arrangement of the spore cushions was noted except that they were formed most frequently in close proximity to some recently dead host plant, and seemed to develop best in moist, shaded places, such as cracks and holes in the ground.

During the summers of 1921 and 1922 two infected alfalfa fields at Sacaton, Arizona, were closely watched for the occurrence of the fruiting forms. Two abundant crops of conidial material appeared in all of the infected spots in 1921, the first following a rainy period during July 23 to 31 and the other following a heavy rain on August 21. There were also two heavy crops and several lighter ones in 1922, the most abundant appearing after a heavy rain on August 31. The abundant crops enabled the writer to observe that the arrangement of fruiting bodies was not haphazard, but that their habit of formation was in definite circles around the ring of plants which had recently wilted.

It was also noted that when conditions were most favorable the mats not only issued from every available crevice and hole in the surface of the ground, but formed upon smooth surfaces. In several spots in August, 1922, the mats were so close together that after two days of centrifugal growth they presented a formation so nearly continuous as to resemble a buff-colored band about 18 cm. wide around the periphery of the diseased area (pl. 2, A).

Thorough search failed to reveal any of the mats away from the infected spots, and it was but rarely that one could be found more than 30 cm. inside the ring of recently wilted plants. Having noted many times that

the spore mats frequently appeared at the mouths of holes in the ground (Pl. 1, B), and desiring to ascertain if the fruiting bodies advanced in a centrifugal manner with the spread of the disease, the writer made a circle of 50 holes with a one-inch soil tube on July 27, 1921, just 15 cm. outside the circle of dying plants, and not closer than 15 cm. to the nearest spore mats. Conditions continued favorable for the fruiting of the fungus and within 10 days spore mats had made their appearance, lining the mouths of 23 of the holes.

On August 25, 1921, a count was made of all the spore mats in an irregular rootrot spot of approximately 500 square meters in area in an alfalfa field at the Cooperative Testing Station, Sacaton, Ariz. The surface

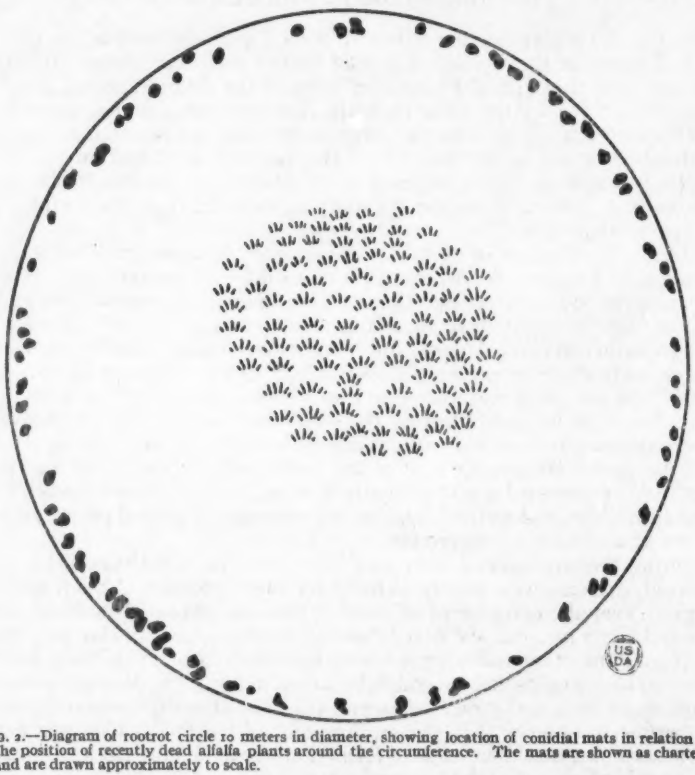


FIG. 2.—Diagram of rootrot circle 10 meters in diameter, showing location of conidial mats in relation to the position of recently dead alfalfa plants around the circumference. The mats are shown as charted, and are drawn approximately to scale.

area of each mat was measured and its location with reference to the perimeter of the spot was charted. Of the 98 mats charted 80 were within 60 cm. of the rim of recently wilted plants, and all but one of the other 18 were old powdered masses of a former crop. The total surface area occupied by the mats was 2.5 square meters.

On September 6, 1922, a circular spot 10 meters in diameter in an alfalfa field on the Indian School farm at Sacaton was charted in the same manner. (See fig. 2 and pl. 2, A.) All of the 71 freshly formed spore bodies were within 40 cm. of the ring of recently wilted plants, and the total surface area occupied by the mats was 1.86 square meters.

EFFECT OF SOIL CONDITIONS ON THE PRODUCTION OF FRUITING BODIES

The production of the fruiting bodies in rootrot spots seems to be somewhat dependent on the soil conditions. In the Salt River Valley no fruiting bodies were observed in infected cotton fields of a sandy nature, no matter how favorable the weather conditions. In 1918, however, fruiting bodies were observed in two instances in infected alfalfa fields in soils of a sandy character. Although found in abundance during the seasons of 1921 and 1922 in the alfalfa fields near Sacaton, where the soils are composed largely of fine sand and silt, fruiting bodies were never seen in infected cotton fields of that region, although diligent search was made during the fruiting periods. The promptness with which the surface of sandy soils dries out after rains, when not covered by some dense crop such as alfalfa, probably explains the absence of the conidial mats in sandy areas. With the exception of one field the sandy areas at the time surveyed did not appear to be seriously infected. Soil samples taken from one of the spots in this badly infected field gave a moisture equivalent of only 3.8, while the soils typical of the largest and most severely infected areas in the Salt River Valley gave a moisture equivalent of 20 to 24. In Table I is given a list of infected fields under the writer's observation in 1918, with their soil type and estimates as to the number of fruiting bodies observed during the summer. The fields varied in area from 2 to 16 hectares, and the infected portions were roughly estimated. It will be observed that there appears to be a fairly close correlation between the presence of the fruiting bodies and heavy, finely divided soils.

TABLE I.—*Location of fields with crops and areas infected with rootrot in 1918, and the type of soil and estimated number of fruiting bodies observed*

Field No.	Crop infected.	Estimated area of field infected.	Estimated fruiting bodies per 100 square meters.	Kind of soil.
		Ares.[a]		
1	Alfalfa	202.3	5	Silt loam.
2	do	44.5	.....	Sand.
3	do	80.8	.....	Do.
4	do	12.1	.....	Do.
5	do	202.3	.....	Sandy loam.
6	do	202.3	3	Clay loam.
7	Cotton	161.8	18	Clay loam—stony subsoil.
8	Alfalfa	20.2	.....	Clay loam.
9	do	80.9	23	Do.
10	Cotton	121.4	6	Adobe—calcareous subsoil.
11	do	202.3	.....	Clay loam.
12	do	80.9	5	Adobe.
13	do	40.5	13	Clay loam.
14	do	80.9	7	Adobe.
15	do	121.4	11	Clay loam.
16	do	16.2	3	Sandy loam.
17	do	202.3	21	Sandy loam—stony subsoil.
18	do	20.2	.....	Sand (coarse).
19	Alfalfa	60.6	.....	Clay.
20	Cotton	80.9	.....	Clay loam.
21	do	40.5	.....	Do.
22	do	12.1	5	Adobe.
23	do	20.2	16	Adobe—stony subsoil.
24	do	40.5	3	Adobe—loamy subsoil.

[a] An are is 100 square meters.

The results of chemical analyses (Table II) of samples of soil taken from these areas seem to indicate that the formation of fruiting bodies is favored by a high content of organic matter. This, however, might be credited to the greater water-holding capacity of such soils. In Table II is shown the percentage of humus and organic carbon determined in soil samples from the fields listed in Table I, from both inside and outside the diseased areas. Composite samples were obtained by mixing the soil from 5 borings taken at various intervals inside the infected spots. A like number of borings were taken about 50 meters distant from any infection to represent noninfected soil. The humus and organic matter were determined from the same air-dried samples. The humus was determined by removing the calcium from 10 gm. of soil with dilute HCl (1 per cent), washing out the chlorids, extracting the soil with 500 cc. of 4 per cent ammonia for 24 hours, and measuring the intensity of the humus color in a colorimeter against a standard humus solution. The organic carbon was determined by boiling 20 gm. of soil with 50 or 75 cc. of a mixture of H_2SO_4 and potassium bichromate, using the larger amount with soils containing more than the average amount of organic matter. The acid mixture was made up in the proportion of 120 gm. of the bichromate to 1,000 cc. of concentrated H_2SO_4. The CO_2 wasa bsorbed in N2/3 NaOH in a bead tower and the whole of the hydroxid solution removed and titrated.

TABLE II.—*Percentages of humus and organic carbon in rootrot spots and in adjoining noninfected areas*

Field No.	Humus, first foot.			Organic carbon, first foot.		
	Infected.	Non-infected.	Difference.[a]	Infected.	Non-infected.	Difference.[a]
1	0.577	0.832	+0.255	0.818	0.883	+0.065
2	.182	.307	+.125	.269	.324	+.055
3	.096	.307	+.211	.246	.324	+.078
4	.207	.307	+.100	.468	.324	−.144
5	.500	.500	.000	.201	.228	+.027
6	.700	.770	+.070	.382	.437	+.055
7	.822	.750	−.072	.127	.427	+.300
8	.286	.276	−.010	.427	.516	+.089
9	.882	.832	−.050	.812	.730	−.082
10	.400	.476	+.076	.490	.617	+.127
11	.182	.267	+.085	.298	.401	+.103
12	.715	.715	.000	.691	.557	−.134
13	.500	.308	−.192	.518	.415	−.103
14	.455	1.120	+.665	.535	.967	+.432
15	.625	.715	+.090	.595	.980	+.385
16	.313	.476	+.163	.286	.360	+.074
17	.587	.682	+.095	.334	.566	+.232
18	.832	1.072	+.240	.571	.622	+.051
19	.625	.625	.000	.310	.648	+.338
20	1.000	1.250	+.250	.758	.832	+.074
21	.860	1.000	+.140	.782	1.094	+.312
22	1.250	1.250	.000	.926	1.085	+.159
23	.939	1.500	+.561	.353	.641	+.288
24	.939	.832	−.107	.797	.893	+.096
Average	.603	.715	+.112±.0258	.500	.620	+.120±.0214

[a] Difference plus indicates the greater percentage in noninfected areas; difference minus indicates the reverse.

It will be noted that in 15 of the 24 fields the percentage of humus is greater in the first foot of soil in noninfected areas than in infected areas. In 20 of the fields the percentage of organic carbon in the samples from the first foot of soil outside the infected areas is greater than the organic carbon content of samples taken from the inside. In reporting studies of chemical changes brought about by the development of mycelium in fungus fairy rings, Shantz and Piemeisel (7) state that such changes consist largely in the reduction of organic matter by the mycelium. Lawes, Gilbert and Warington (3) call attention to the fact that in their investigations the percentage of carbon was found to be uniformly higher outside the ring than on the periphery or within the ring. Although the occurrence of less organic matter inside the majority of rootrot areas than outside may have no significance, the similarity of the behavior of this parasite to the habits of the fairy ring fungi seems to make this fact worth recording. No effort was made to determine if the mycelium and fruiting bodies played any part in the breaking down of the organic matter of the soil into ammonia or other compounds, but an analysis of the powdered fruiting bodies showed an organic nitrogen content of 1.73 per cent.

EFFECT OF WEATHER CONDITIONS ON THE FORMATION OF FRUITING BODIES

The production of fruiting bodies seems to be almost entirely dependent on weather conditions. During periods of dry, hot weather freshly formed conidial mats were never observed, no matter how frequently irrigation water was applied nor how heavy the type of soil.

In Table III is shown the precipitation at Phoenix and Sacaton during the months of fruiting activity in 1917 to 1922, inclusive, and the relative number of fruiting bodies noted in the fields visited. It will be observed that the fruiting occurred in most cases during the months of heaviest rainfall. The apparent exception in July, 1922, was evidently influenced by the prolonged period of light showers and high humidity occurring from the 15th to the 20th. During the month of August, 1920, when the precipitation was 0.89 inch, the rainy periods were never as prolonged.

TABLE III.—*Monthly precipitation during fruiting season at Phoenix, Ariz., 1917 to 1919, and at Sacaton, Ariz., 1920 to 1922, and estimated number of fruiting bodies observed in infected fields of these localities*

	1917		1918		1919		1920		1921		1922	
	Precipitation.	Mats per 100 square meters.	Precipitation.	Mats per 100 square meters.	Precipitation.	Mats per 100 square meters.	Precipitation.	Mats per 100 square meters.	Precipitation.	Mats per 100 square meters.	Precipitation.	Mats per 100 square meters.
	Inches.		*Inches.*		*Inches.*		*Inches.*		*Inches.*		*Inches.*	
June	0.15	.....	0.08	.....	Trace.	.....	0.23	.....	Trace.	.....	0.11	.....
July	3.97	13	1.02	8	1.05	19	0.48	.....	2.41	17	0.53	23
August	0.11	.....	3.47	10	2.40	7	0.89	.....	3.26	24	3.32	42
Sept	0.55	.....	0.39	.....	1.93	.....	0.21	.....	0.39	.....	0.55	[a] 40

[a] Formed the first week in September, presumably from the effect of 1.56 inches of rain August 31. This is the only instance noted where fruiting occurred in September.

The periods of most active fruiting usually lasted only two or three days unless followed by continued rainy or humid weather. A rainy period began on July 23, 1921, and continued for about 10 days. During this period the mats developed in such abundance as to cover almost the entire surface of the ground around the circles of dying alfalfa plants in some fields near Sacaton. Jorden (*2*) and Shantz and Piemeisel (*7*) have called attention to the influence of weather conditions, especially moisture, on the production of fruiting bodies in fungus fairy rings. It would appear that the organism associated with rootrot in Arizona is no less sensitive to this influence in its processes of fructification.

REESTABLISHMENT OF ALFALFA PLANTS IN ROOTROT AREAS

In studying the behavior of rootrot in alfalfa fields, the writer observed that a considerable number of alfalfa plants recover even after the portions above ground have succumbed to the rootrot attack. When the circles, which begin with one dead plant and spread in all directions, have attained a diameter of 3 or 4 meters, various weeds and grasses begin to appear in the center of the bare circle and spread outwardly (pl. 2, B), rarely reaching within a distance of 1 meter of the rim of dying plants during the time in which the disease is most active. The plants which appear most frequently in the alfalfa rootrot circles near Sacaton are *Capriola dactylon, Holcus halepensis, Echinocloa colona, Chenopodium murale, Trianthema portulacastrum, Leptilon canadense, Leptochloa imbricata, Physalis angulata.* Starting at about the same time as the weed growth, a number of alfalfa plants near the center of the circle begin to send out new growth. When water is applied through rainfall or irrigation it often happens that a great number of these supposedly dead plants begin to leaf out, and, along with the weeds, revegetate the central portion of the bare circle. When one of these plants is lifted from the ground, it is often found that a piece of taproot, about 15 or 20 cm. long, has remained alive, just below the crown from which a number of lateral roots have extended, and brought new life to the plant (pl. 3, B). In rare instances plants are found which show the entire taproot rotted away and a large number of lateral roots branching out directly under the crown. In several circles the number of reestablished plants were counted, and it was found that they ranged from none in small circles, of about 2 meters diameter, to 38 per cent in larger circles, about 20 meters in diameter. During the winter months circular rootrot spots are often observed whose entire areas are occupied by several concentric circles of reestablished plants interspaced alternately with bare circles where no alfalfa growth was renewed. It is evident that the rings of plants represent those which had died just before a summer cutting of hay which was immediately followed by the customary irrigation. The bare rings doubtless represent the location of plants which died when the soil was comparatively dry, and to which no water was applied for a considerable time.

MANNER OF RECURRENCE OF ROOTROT SPOTS DURING SUCCESSIVE YEARS

Scofield (*6*), from charting rootrot spots in rotation experiments at the San Antonio Experiment Station for 7 years (1912 to 1918), has shown that while the disease in cotton fields usually occurs in well-defined spots in one season it may not recur in the same places in the

following season, but may appear elsewhere. Some observations were made at Sacaton during the seasons 1920 to 1922 to determine if this was also a characteristic behavior in the case of a perennial host plant such as alfalfa. The infected spots in a 9-hectare alfalfa field adjoining the Experiment Station at Sacaton were measured and charted on September 30, 1921, when the disease was approaching a state of quiescence. In the early part of the summer of 1922 these spots were observed closely, so as to note the points of renewed infection. The first dead plants were noticed on June 11, and in nearly every instance the first plant

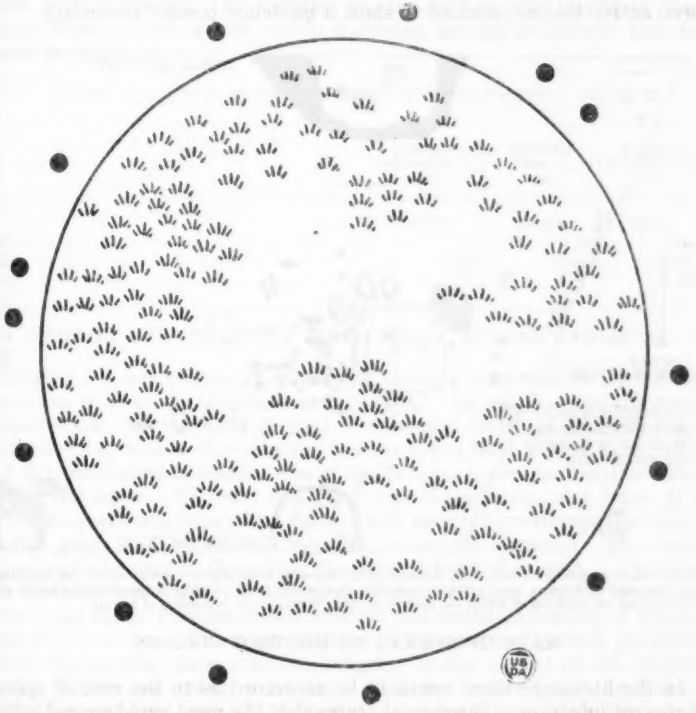


FIG. 3.—Location of initial points of infection in relation to formerly occupied area at the beginning of the seasonal activity of rootrot fungus. The same area as shown in Plate 4, A. The new centers and reestablished plants are shown as actually located in the field and are drawn to approximate scale.

showing infection was located just outside the periphery of the previous season and not over 70 cm. from it (fig. 3). Almost simultaneously with the wilting of the first plant other plants located at various intervals outside the old periphery, but always at the same distance from the center of the old infected area, showed infection (pl. 4, A, fig.3). Each of these plants served as a center for the infection of the plants nearest to them, but the many small infected areas (appearing at the same distance from the old periphery) soon merged into a regular band completely circumscribing the old area of the previous year (pl. 4, B). The

infection then spread centripetally as well as centrifugally, and it frequently happened that the majority of the plants reestablished inside the old areas during the fall and winter were killed by this retroactive invasion.

The map shown in Figure 4 shows the location of activity in the 9-hectare field during the seasons of 1921 and 1922. It will be noted that with the great majority of rings the diseased area of the succeeding year seems to be merely an extension of the centrifugal spread of the previous season. Some of the spots, however, showed no renewed infection in 1922. The largest areas, which showed indications of having been active longest, seemed to show a tendency toward immunity.

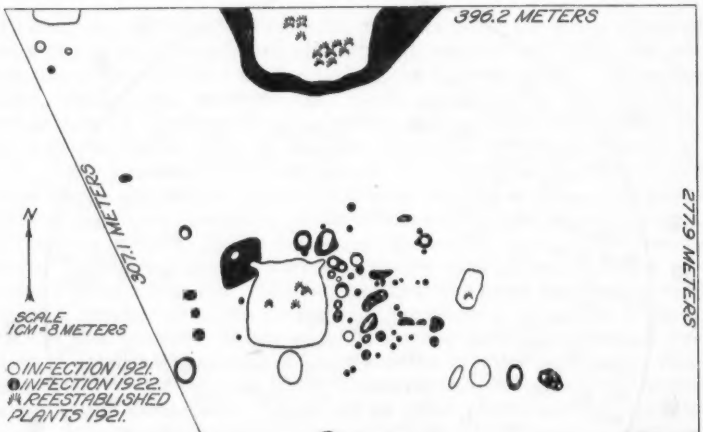


FIG. 4.—Map of 9-hectare alfalfa field, Sacaton, Ariz., showing areas infected with rootrot on September 30, 1921, and on June 22, 1922. Note the circular form maintained by most of the isolated centers of infection and the position in which the disease activity recurs at the beginning of season.

RATE OF SPREAD OF ROOTROT CIRCLES

In the literature there seems to be no record as to the rate of spread of rootrot infection. Duggar (*1*) states that the most rapid spread which he observed was in a field of irrigated alfalfa, and Pammel (*4*) notes that areas of considerable extent in cotton fields may be affected in one season; but no actual measurements are recorded. On September 30, 1921, the diameters of three circular spots in an alfalfa field were measured, and photographs made of the areas. They were later measured on June 26 and September 30, 1922. Photographs were taken of the three spots on the dates named in the legends for Plates 4, 5, and 6, and approximate measurements made. The rates of spread of the three areas are given in Table IV. It will be observed that the greatest spread was in circle No. 2, where the increase in diameter was 14.4 meters in one year, but this was caused by its merging with a neighboring spot.

It was frequently observed that the rate of advance in cotton fields was greater than in alfalfa fields. On August 21, five cotton plants, located at least 20 meters apart, were found dying from rootrot in a field where infection had never been seen. At the time when first observed in a wilted condition there was no evidence of any other rootrot infection within 25 meters. Only two of the spots resulting from these centers of infection maintained the regularity of their circular margins throughout the remainder of the season; the other three became irregular because of new centers of infection appearing nearby and merging with them as they spread. By October 10, the two regular circles of dead plants were 9.1 and 9.4 meters in diameter, having progressed this distance in 50 days.

TABLE IV.—*Rate of spread of rootrot circles during one year of activity*

Circle.	Diameter September 30, 1921.	Diameter June 22, 1922.	Diameter September 30, 1922.
	Meters.	*Meters.*	*Meters.*
1	7.0	9.1	14.6
2	8.8	12.2	23.2
3	4.6	8.8	12.8

RESEMBLANCE TO OTHER FUNGI WHICH SPREAD RADIALLY

Duggar (*1*) has compared the radial manner of spread of the rootrot organism to that of "*Rhizoctonia crocorum*." In many ways the aerial behavior can be compared to that of the fungi which cause fairy rings. The rate of spread is of course much more rapid than that of fairy rings, but the manner of spreading centrifugally from a central point of infection is the same. Ritzema Bos (*5*) likens the advance of a fairy circle to the progressive advance of a flame which results from dropping a match in dry grass and which will spread continually outward. No better figure could be used in describing the field activity of rootrot. Attention has already been called to the resemblance between rootrot areas and fairy rings in the manner of the renewal and radial expansion of vegetation at the center. The outer margin of the renewed growth gradually advances, keeping pace with the progress of the border of the original circle. There are many indications that the interiors of the rootrot circles become free from the disease at least for a year after the attack has passed on. Occasionally one of the recovered plants will die, and the infection will spread to neighboring plants, but this behavior is not common. In 1918 the writer had under observation several diseased spots of Hairy Peruvian alfalfa in which the inside of the areas had been replanted with Grimm alfalfa. During the entire season none of the plants of the Grimm variety showed any infection. On July 12, 1922, the spot shown in Plate 6, A, was spaded up and planted to cowpeas. No rootrot infection occurred during the season, and the cowpeas grew luxuriantly (pl. 3, A).

A characteristic of rootrot is that the fruiting bodies occur only near the margin of the diseased circle. This seems to be a further indicati n that the disease is not active except near the rim of the circle (pl. 6, D). It is not the purpose of the writer to try by this comparison to establish

the relationship of the rootrot fungus to the hymenomycetes, but it is hoped that this characteristic of the organism, heretofore not described may prove of some significance to taxonomists who may become interested in the disease. There seems to be sufficient evidence to indicate clearly that the organism belongs to the basidiomycetes.

Although technical proof is still lacking that the conidial material is the true fruiting form of Shear's *Ozonium omnivorum*, there can be little doubt that it plays an important part in the life of that organism. Its frequent occurrence and conspicuous appearance under Arizona conditions makes it an important and easy means of establishing the identity of rootrot activity when that is likely to be confused with the effect of alkali salts, lightning, or rodent injuries. The presence of the mats on fallow or virgin lands should be sufficient indication of the risk of planting susceptible tree crops or other plants of a permanent nature.

POSSIBLE CONTROL OF ROOTROT IN ITS INCIPIENT STAGE

The resemblance of the manner in which rootrot spreads to the advance of fungus fairy rings suggested to the writer the possibility of some barrier treatment whereby the infected areas might be isolated and their advance checked. Several methods of treating small areas with formaldehyde were attempted in September, 1921, and June and July, 1922, but these were only partially successful. On August 3, 1922, three small infected areas about 1½ meters in diameter in an alfalfa field were segregated from the remainder of the field by throwing up circular dikes of soil 45 cm. in advance of the circle of dying plants, and the soil within was saturated to a depth of 1.2 meters by pouring in a solution of formaldehyde (1 part 40 per cent formalin to 100 parts water). Three control areas were also designated, and the limits of the disease indicated by small stakes or by dikes of soil. On August 19, photographs were taken of one treated and one control area (pl. 7) to show the difference in the rate of spread 16 days after treatment. The average increase in the diameter of the control areas was 1.1 meters, while the treated areas showed no dead plants outside of the treated circle. On September 30, the control areas had increased by an average of 3 meters, while the treated areas showed no dead plants outside the dikes (pl. 7).

On August 21, eight small areas of infection, each consisting of only one or two dead plants, were selected in a cotton field in which the spots were just beginning their seasonal appearance and were yet 25 or 30 meters apart, and were treated as above described for the alfalfa areas except that the dikes were thrown up about 90 cm. outside of the infected plants. It was found necessary to extend the treatment to this distance in order to include all infected roots. On September 30 no other plants had died from the disease on the periphery of these spots, although three of the areas had to be disregarded because of new spots beginning near by and invading the soil contiguous to the treated areas. Five control areas were located, only two of which maintained their circular form; these had increased in diameter 9.1 and 9.4 meters respectively.

It was observed that healthy alfalfa and cotton plants were seriously affected by the formaldehyde, even when diluted to 1 part formalin in 400 of water, but after shedding their leaves the plants soon recovered and resumed growth. There was no evidence that the soil fertility was at all impaired by the formalin solution used in the extermination of the

rootrot mycelium. In a few weeks following the treatment weed growth became profuse within several of the treated areas.

While it is not considered likely that such a method as is here described will be commonly practiced, because of the labor and expense involved, it seems to hold out promise of further success in the effort to master this destructive parasite. Even without modifications, this method might have possibilities for use on especially valuable land such as city property, experiment farms, and bearing orchards, where the limits of the diseased spots could be defined by planting some kind of leguminous cover crop

SUMMARY

(1) Rootrot, a disease caused by *Ozonium omnivorum*, is becoming more and more serious in the Salt and Gila River Valleys of Arizona, where, owing to the extension of the cotton industry in recent years, it is found to be more widespread than was formerly thought when alfalfa was the principal crop.

(2) In alfalfa fields it is the habit of the disease to spread radially and to form almost perfect circles, the more recent activity being defined by the ring of recently wilted plants on the circumference.

(3) Conidial mats such as have been described by Duggar have been observed closely associated with the disease for six seasons in the vicinity of Phoenix and Sacaton, Ariz.

(4) With but few exceptions, a characteristic behavior not heretofore described is that the conidial mats appear only in close proximity to the plants that have most recently succumbed to the disease.

(5) The chief requisites for fructification of the fungus appear to be a heavy type of soil with a dense cover crop and humid weather with intermittent rainfall.

(6) In the majority of fields examined the amount of humus and organic carbon was less per surface foot of soil inside of rootrot areas than in adjacent areas not infected.

(7) Diseased areas in alfalfa fields become occupied by various kinds of weeds and alfalfa plants, originally thrifty, which have recovered by sending out adventitious roots from fragments of old taproots that have remained alive just beneath the crown.

(8) In some seasons many of the larger spots show no renewed infection, but in the case of most of the smaller spots the diseased area of one year seems to be merely an extension of the diseased area of the previous season.

(9) The rate of enlargement of regular circles in an alfalfa field was about 8 meters increase of diameter per year. In cotton fields regular circles increased in diameter about 9 meters in 50 days.

(10) The behavior of rootrot in alfalfa fields resembles that of fungus fairy rings in their manner of spreading radially, in areas becoming free from the disease after the active mycelium has passed on, and in the formation of fruiting bodies about the ring of most recent activity.

(11) In Arizona the fruiting form provides an easy means of establishing the identity of rootrot activity.

(12) A method of controlling the disease by promptly providing barriers for segregating new centers of infection, and saturating soil around them with formaldehyde solution was tried and found effective, and points to the possibility of some more practicable means of control.

LITERATURE CITED

(1) DUGGAR, B. M.
1916. THE TEXAS ROOTROT FUNGUS AND ITS CONIDIAL STAGE. *In* Ann. Mo. Bot. Gard., v. 3, p. 11–23, 5 fig. Bibliography, p. 23.

(2) JORDEN, George.
1862. SOME OBSERVATIONS ON FAIRY RINGS, AND THE AGARICS THAT PRODUCE THEM. *In* The Phytologist, n. s., v. 6, p. 236–238.

(3) LAWES, J. B., GILBERT, J. H., and WARINGTON, R.
1883. CONTRIBUTIONS TO THE CHEMISTRY OF "FAIRY RINGS." *In* Jour. Chem. Soc. [London], v. 43, p. 208–223.

(4) PAMMEL, L. H.
1890. COTTON ROOTROT. Tex. Agr. Exp. Sta. Bul. 7, 30 p., 5 pl.

(5) RITZEMA BOS, Jan.
1901. "HEKSENKRINGEN," "KOL" OF "TOOVERKRINGEN," "DUIVELS KARNPAD" OP WEILANDEN. *In* Tijdschr. Plantenziekten, jaarg. 7, p. 97–126.

(6) SCOFIELD, C. S.
1919. COTTON ROOTROT SPOTS. *In* Jour. Agr. Research, v. 18, p. 305–310, 7 fig.

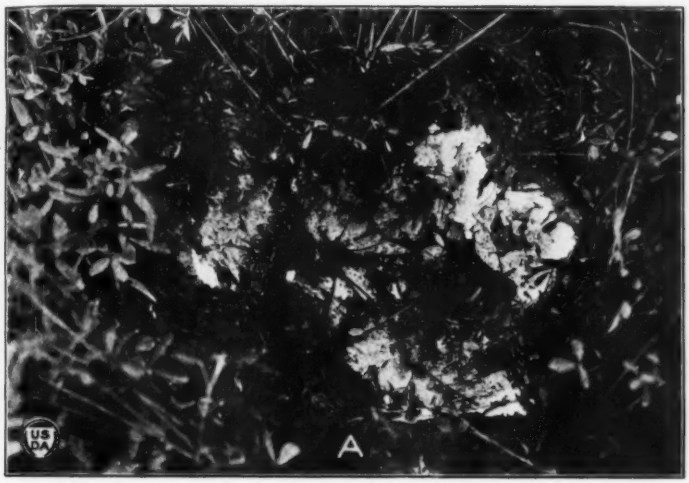
(7) SHANTZ, H. L., and PIEMEISEL, R. L.
1917. FUNGUS FAIRY RINGS IN EASTERN COLORADO AND THEIR EFFECT ON VEGETATION. *In* Jour. Agr. Research, v. 11, p. 191–246, 15 fig., pl. 10–30. Literature cited, p. 242–245.

(8) SHEAR, Cornelius Lott.
1907. NEW SPECIES OF FUNGI. *In* Bul. Torrey Bot. Club, v. 34, p. 305–317.

PLATE 1

A.—A conidial mat 1 day old. Still increasing its area by active growth on the periphery where the pronounced white color is shown. The stromatic mycelium which spreads 1 or 2 cm. in advance of the conidiphores is identical with the characteristic Ozonium form on the roots of infected plants.

B.—Conidial mats forming in holes made by rodents. Mats on the inside of gopher runways have been observed to remain well preserved for a month after the last of the fruiting bodies on the surface of the ground had been disintegrated by weathering agencies.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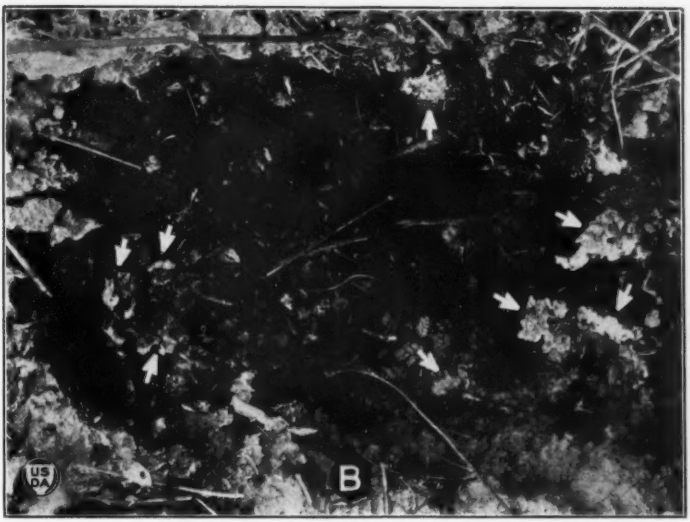
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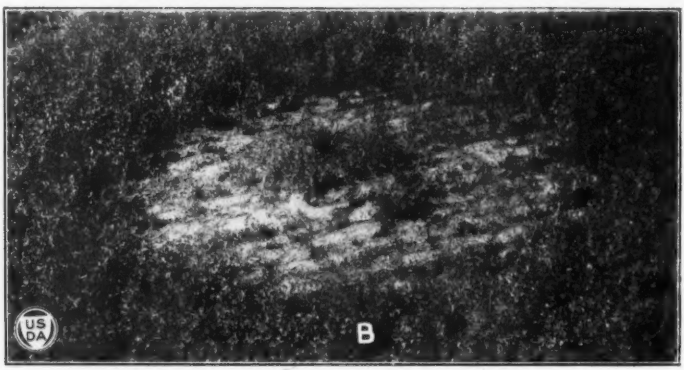
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PLATE 2

A.—Conidial mats in almost continuous formation around the periphery of a rootrot circle. The mats were given a light coating of whitewash solution to accentuate the white color for photographing. September 6, 1922.

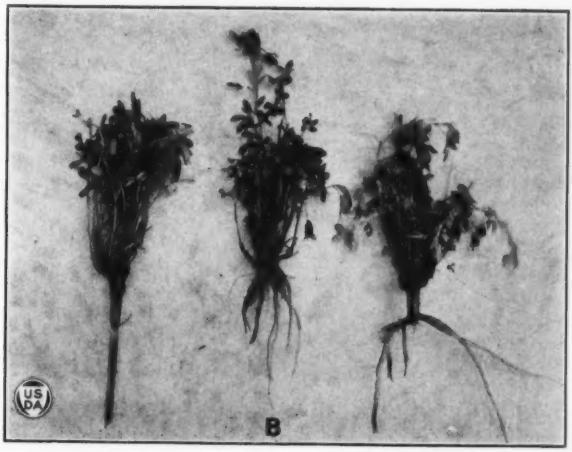
B.—Weed growth and recovered alfalfa plants beginning to appear in the center of a rootrot circle. September 6, 1922.

PLATE 3

A.—Cowpeas occupying the center of a rootrot spot while alfalfa plants continue dying on the outer edge. The mycelium advances at the rate of 1 or 2 meters per month. Cowpeas are very susceptible to rootrot.

B.—Manner in which alfalfa plants recover from rootrot.

A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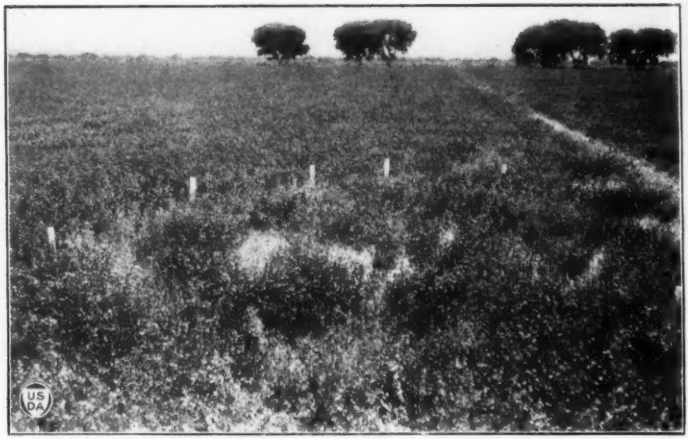

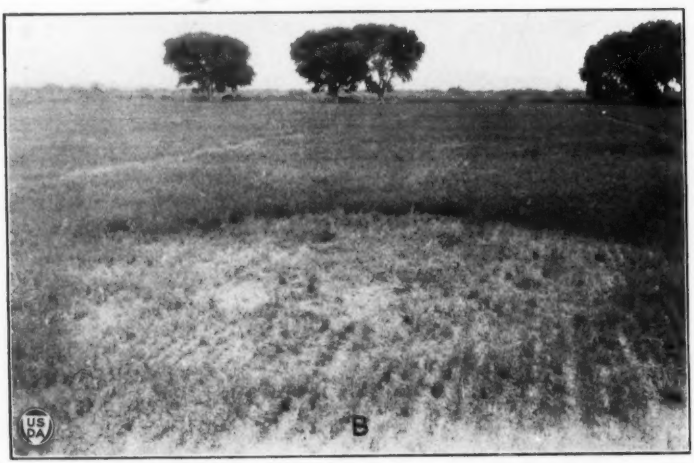
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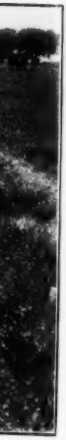


D. C.

PLATE 4

A.—Recurrence of rootrot infection at the margin of an old area 8.8 meters in diameter, June 18, 1922.

B.—The same circle July 6, 1922, 10 meters in diameter.

PLATE 5

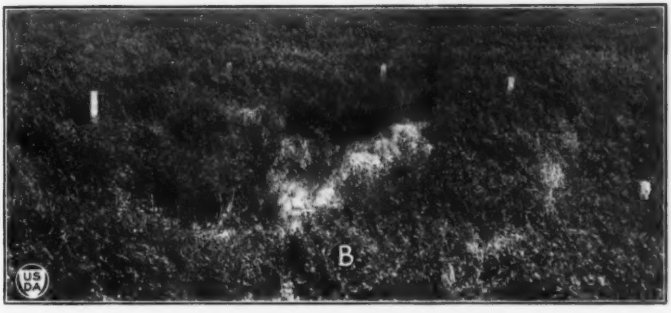
A.—Rootrot spot, approximately 7 meters in diameter, in an alfalfa field, September 18, 1921.

B.—The same spot at the beginning of disease activity, June 18, 1922, now approximately 9 meters in diameter. The stakes designate the outer rim of the circular band of dying plants.

C.—The same spot July 6, 1922, after the hay had been cut.

D.—The same spot August 26, 1922, approximately 13 meters in diameter. In the interior of the spot appears the growth of grass, weeds, and reestablished alfalfa plants. Light colored fruiting bodies of the fungus can be seen at the right, near the periphery.

A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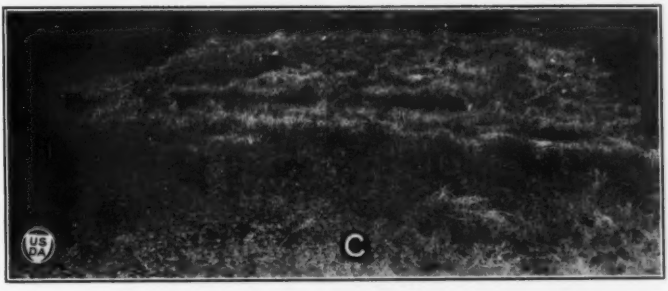
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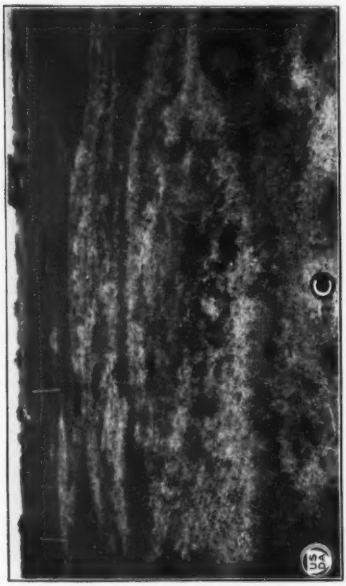

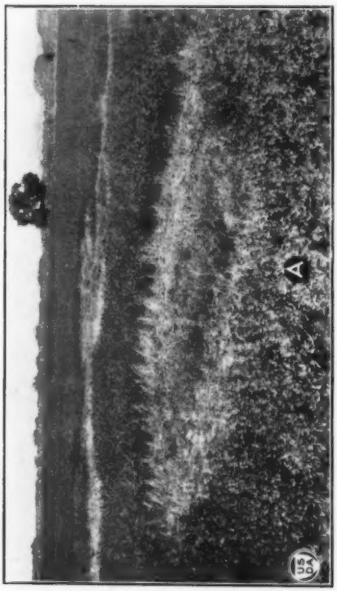

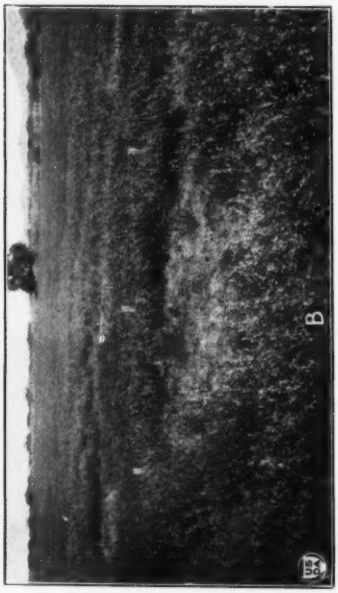

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PLATE 6

A.—Rootrot spot, approximately 7 meters in diameter, in an alfalfa field, September 18, 1921.

B.—The same spot June 18, 1922, at the beginning of seasonal activity, approximately 11 meters in diameter.

C.—The same spot July 6, 1922, at the point of conjunction with another infected spot.

D.—Rootrot circle 18 meters in diameter in alfalfa field, Sacaton, Ariz., 1923. Conidial mats several days old are shown near the periphery, and the zone of active disease has advanced beyond them. A whitewash solution was applied to the mats to facilitate photographing. The white stakes indicate the furthermost margin of active disease the previous year.

PLATE 7

A.—Segregated rootrot area 16 days after treatment with formaldehyde. No plants outside the original area had died since treatment.

B.—Control area of approximately the same original size; the disease had advanced 61 cm. in 16 days.

C.—The rootrot area shown in Plate 7, A, 60 days after treatment. No further infection had been observed.

D.—The control area shown in Plate 7, B, 60 days after treatment of the infected spot in A. The disease had increased the diameter of the circle by about 3 meters. In order to make it more comparable to the treated area, a dike was thrown up about the stakes which were placed to define the limits of the disease on August 3.

THE THREE-BANDED GRAPE LEAFHOPPER AND OTHER LEAFHOPPERS INJURING GRAPES[1]

By G. A. RUNNER, *Entomologist*, and C. I. BLISS, *Collaborator, Fruit Insect Investigations, Bureau of Entomology, U. S. Department of Agriculture*

INTRODUCTION

Studies of the leafhopper fauna on grape, commenced in 1919, have reached a stage where a preliminary report seems warranted. A survey of the literature shows that all five subfamilies of the Cicadellidae occurring east of the Rocky Mountains have been reported on grape, as follows: (1) Bythoscopinae, 1 species; (2) Gyponinae, 1 species; (3) Cicadellinae, 4 genera, 5 species; (4) Jassinae, 2 genera, 3 species; (5) Typhlocybinae, 6 genera, 34 forms, mostly distinct species.

SYSTEMATIC RELATIONSHIPS OF FORMS STUDIED

Although the writers' collections extend from the Hudson River Valley section to the Lake Michigan grape belt, greatest emphasis has been laid upon the Sandusky, Ohio, region. Here most of the hoppers belong to the genus Erythroneura. Their systematic relationships within the genus are obscure, but an examination of the internal genital apparatus in connection with nymphal characteristics promises a solution. So far as this work has progressed, the following scheme for the forms in the Sandusky region may be tentatively adopted:

(1) *Erythroneura tricincta* var. *cymbium* McAtee: On the whole the most injurious and most common form in commercial grape-growing centers west of Cleveland.
(2) *E. comes* var. *compta* McAtee: In large portions of the Lake Erie Island grape belt of greater economic importance than *tricincta*.
(3) *E. comes* var. *comes* Say: The traditional "grape leafhopper," of importance chiefly on the mainland east of Sandusky.
(4) *E. vitifex* Fitch: Difficult to separate from *comes* except by dissection, hence of doubtful distribution, although apparently common in the Lake Erie Island region, especially on thin-leaved grapes.
(5) *E. vulnerata* Fitch: Very widely distributed, but usually unnoticed because of its different habits.
(6) *E. ziczac* Walsh: Common on thin-leaved grapes throughout the belt.
(7) *E. vitis* Harris: Restricted to thin-leaved grapes and dominant on wild *Vitis vulpina*.
Eight additional forms also occur in the Sandusky region, usually in smaller numbers and chiefly on wild grape.

ECOLOGICAL RELATIONSHIPS

Detailed studies on the ecological relationships of these forms show marked differences in the density and composition of the hopper population between different varieties and species of grape and between different regions of varying size. These problems will be considered in a special paper.

Erythroneura tricincta var. *cymbium*, in the regions of its greatest abundance, comprises a larger proportion of the hopper population on thick-leaved grapes such as Concord and Catawba than on thin-leaved grapes such as Delaware, Clinton, and wild *Vitis vulpina*. Other varieties

[1] Accepted for publication October 2, 1923.

of *E. tricincta*, notably *tricincta* Fitch and *calycula* McAtee, show exactly the opposite habit, occurring rather commonly on wild *Vitis vulpina*, Clinton, and other thin-leaved cultivated grapes, but being practically absent from varieties such as Concord and Catawba. Varieties *tricincta* and *calycula*, in both of which the anterior cross-band overlaps the base of the scutellum, intergrade; are very much more widely distributed geographically than *cymbium*, with known range including Connecticut, Tennessee, and Kansas; and occur in much smaller numbers than *cymbium*. This ecological difference between *cymbium* and *tricincta-calycula* explains the apparent contradiction between our results and those reported by Hartzell (*3, p. 35–36*)[2] in 1912.

THE THREE-BANDED GRAPE LEAFHOPPER

This discussion will be restricted to a single form, *Erythroneura tricincta* var. *cymbium*, because of its great economic importance. Although the common name "three-banded grape leafhopper" is applicable to several other forms of *tricincta* of less economic importance, it is here adopted for this insect, being the name applied to it by grape growers throughout its range. The term "three-banded" is used by Fitch (*2, p. 63*) in the original description of *E. tricincta*. (*E. tricincta* var. *tricincta* is doubtless the typical variety of Fitch.)

ECONOMIC IMPORTANCE

Though not especially abundant in 1919, *Erythroneura tricincta* var. *cymbium* caused severe injury in 1920 and was about as numerous and destructive in 1921 and 1922. In numerous localities on the Lake Erie Islands and in the Sandusky region in 1922 the crop loss in Catawba vineyards was unusually heavy, as a large part of the fruit on injured vines failed to ripen properly. Well ripened Catawba grapes of good quality brought prices ranging from $100 to $140 per ton, while "pale" or poorly ripened grapes sold as low as $40 per ton.

DISTRIBUTION

While common along the southern shore of Lake Erie west of Cleveland, Ohio, the form *cymbium* is infrequent to the east, not extending more than 10 miles east of Cleveland. It is most abundant in the Sandusky region and in the southwestern Michigan grape belt. Our own collecting does not extend west of Benton Harbor, Mich., where it is very abundant and injurious, nor north of Grand Rapids, Mich., where this form is less abundant than *Erythroneura comes*. In Ohio, the form apparently does not extend as far south as Columbus, although just where it stops to the southward has not been determined.

A critical survey of the literature of this variety, judging from descriptions included in general reports on leafhoppers, shows it doubtfully present in Kansas (Lawson, *5*). McAtee (*6, p. 310*) records it from Michigan, from Dallas, Tex., and from Northeast, Pa. The last record, however, represents material originally secured in Ohio and used by Johnson (*4, p. 10–11*) in 1912 for life-history studies. As no specimens of *cymbium* could be found in the vineyards nearest Johnson's old insectary in 1922, the form apparently did not establish itself in the region. The personal collections of Prof. D. M. DeLong at Ohio State University contain specimens of *cymbium* taken at Clarksville, Tenn. W. A. Ross

[2] Reference is made by number (italic) to "Literature cited," p. 424.

of the Canada Department of Agriculture, entomological branch, informs the writers that *cymbium* is the predominant form of leafhopper in vineyards in the St. Catherines and Niagara River district of the Province of Ontario.

DESCRIPTION OF ADULT

The species *tricincta* is separated from other members of the genus Erythroneura by its pale yellow color (during the summer) and three dark cross-bands, the anterior one upon the pronotum, the second one just forward of the middle of the elytra, and the posterior one covering the elytral apices. Variety *cymbium* (pl. 1) differs from other members of the species in that the first cross-band is typically restricted to the pronotum and is profoundly emarginate anteriorly, leaving much of the disk uncolored. Especially under winter coloration, specimens with forward-flexed pronotum may show dark pigmentation upon the anterior border of the scutellum. In no case, however, does this pigmentation overlap completely or evenly a line connecting the humeral angles.

SEASONAL COLOR CHANGES.—In common with other forms of Erythroneura, there are marked seasonal changes in coloration, the color markings of the adults being more distinct and of a deeper tint during the colder months. The bright red longitudinal color streaks on the elytra, so conspicuous during the hibernating period, become pale yellow and indistinct after the overwintering adults appear on the grape foliage in spring. In summer the ground color of the elytra is creamy white or ivory tinted, without the pinkish tinge of fall or winter. The longitudinal color streaks when they first appear on newly transformed adults are pale yellow. During the latter part of August these markings become more pronounced, and gradually change from pale yellow to red as the season advances. The anterior cross-band changes from a velvety reddish brown to a shiny dark brown almost black. The eyes become darker and concolorous with pronotum, the vertexal vittæ reddish and more distinct. The spot at the apex of the scutellum becomes bright red; the two larger spots at the base of scutellum, sometimes fused, yellowish or pale brown. Winter coloration is fairly complete by the first week in December.

LIFE HISTORY AND SEASONAL HISTORY

In its main characteristics, the three-banded hopper has about the same life history as the traditional grape leafhopper (*Erythroneura comes* var. *comes*), i. e., it winters over in the adult stage in or near the vineyards, it attacks the grape foliage in the spring, feeding and breeding on the underside of the leaves, and it appears to pass through two or possibly three generations before frost drives it into hibernation. The hoppers enter hibernation with the sexes about equal in number, but after mating and migrating to the grape foliage in the spring most of the males die, counts early in June showing about 90 per cent females. Overwintering adults lived in confinement the past season well into August.

OVIPOSITION.—The eggs are deposited just beneath the epidermis on the lower surface of the older grape leaves.

PERIOD OF INCUBATION OF FIRST BROOD.—The average incubation period of first-brood eggs in 1922 was 13.7 days, with an average mean daily temperature of 73.9° F., the maximum and minimum incubation periods being 17 and 11 days, respectively. As the first appearance of newly hatched nymphs in numbers occurred June 20, in vineyards in the

vicinity of Sandusky, Ohio, oviposition probably began during the first week in June.

THE NYMPHS.—The nymphal period is passed on the under surface of the grape leaves, the young feeding in the same manner as the adults by sucking juice from the leaf cells. The young move about but little during the developmental period, remaining for the most part on the leaves on which they were hatched. In common with *Erythroneura comes*, the three-banded hopper passes through five nymphal instars, as shown in the accompanying figures. (Pl. 2, A–E.)

NYMPHAL PERIOD OF FIRST BROOD.—The average length of the nymphal instars for first-brood individuals in 1922 follows: First instar, 3.8; second, 3.0; third, 3.4; fourth, 4.4; fifth, 5.4 days, with an average total of 20 days (average mean daily temperature, 72.8° F.; maximum nymphal period, 22 days, minimum, 17 days). As the nymphs migrate very little from leaf to leaf, it is possible to rear them on unprotected growing grape leaves in the insectary, merely wrapping the petioles with absorbent cotton to protect against spiders and contamination. This avoids an abnormal humidity, a factor that has not always been eliminated in previous work.

APPEARANCE OF ADULTS IN SUMMER.—The appearance of first-brood adults in vineyards is determined by the presence of mating pairs. The first pair was found on July 2, and the last on August 31, in 1922, although it can not be stated positively that the last ones were not of the second brood, since the existence of a third brood is still an open question. Mating pairs are most frequent in early morning.

PERIOD OF INCUBATION OF SECOND BROOD.—The incubation period of second-brood eggs, laid between July 26 and 31, 1922, averaged 14.7 days, or one day longer than the earlier generation. The average mean daily temperature of 71.4° F. as compared with 73.9° F. for the earlier brood probably explains this difference. The maximum and minimum incubation periods were 17 and 12 days, respectively.

NYMPHAL PERIOD OF SECOND BROOD.—The developmental period of the second brood varied from 20 to 24 days, averaging 21.8 days, with an average mean daily temperature of 72.4° F. The nymphal instars follow: First, 3.4; second, 3.0; third, 3.8; fourth, 4.6; fifth, 6.9 days. Nymphs were present in vineyards until the second week in October, although in relatively small numbers after the third week in September.

LEAF SURFACE DESTROYED DURING NYMPHAL PERIOD.—In common with other leafhoppers, feeding injury is indicated upon the leaves by well-defined whitened areas. In order to ascertain the leaf surface destroyed during the nymphal period, five grape leaves upon each of which a single hopper had undergone its entire nymphal development were measured. The feeding marks were first drawn by camera lucida or by Bausch and Lomb microprojector at an enlargement of 15 to 24 diameters. The area as drawn was then measured by a planimeter and reduced to square centimeters. There are a number of sources of error in these preliminary measurements, the chief being in the use of dried leaves. This introduces the element of leaf shrinkage, which on comparing the one fresh and four dried leaves measured was found to exceed 16 per cent. Four dried leaves averaged 0.854 square centimeters of leaf surface destroyed by a single leafhopper in reaching maturity.

CHARACTER OF INJURY.—The character of injury caused by the three-banded leafhopper does not differ from that found by previous workers on *Erythroneura comes*. Reduction of functional leaf-surface from removal of cell sap by adult or nymph and probably from attendant injury to sur-

rounding tissue checks growth of wood and fruit, weakening the vine. Fruit from badly infested vines shows the typical inferiority; it does not ripen properly, is poorly colored, low in sugar content, and stained with excreta. When the wood is not normally ripened, part of the buds or whole bearing canes may winter-kill. Although the winter of 1922–23 was comparatively mild, minimum temperatures having been above zero in the Sandusky district, instances of serious freezing back of bearing canes were observed in several northern Ohio vineyards which had been heavily infested with leafhoppers in 1922. This injury was apparently due to a combination of causes—immature wood, caused by leafhopper injury and a heavy crop of fruit (which ripened poorly), and lack of moisture in the soil before freezing, the fall months having been unusually dry.

HIBERNATION.—After the first heavy frosts the adults move about in search of food and cover, congregating in great numbers on sheltered vines where the grape foliage is still green, and remaining as long as food can be obtained. Many travel to surrounding fields or borders, which furnish especially favorable hibernating conditions during winter. During the fall migration, if it can be so called, the species shows a marked tendency to congregate about near-by buildings and in localities protected from wind. Adjacent vineyard areas are commonly centers of heavy infestation during summer. A heavy migration commenced in 1922 on October 13, after the first killing frost. Adults were numerous on grape foliage in sheltered places as late as the third week in November. Vast numbers, in some localities apparently the greater proportion, remain in the vineyards under the vines, where they can be found at any time during the winter. They are generally distributed over the vineyards under partly decayed moist grape leaves in depressions in the ridge of earth under the trellis. On January 29, 1923, between 600 and 700 live adults were obtained from an area of 1 square foot under a Concord vine in the center of a large vineyard, but on the same day only a few specimens could be found in an adjoining alfalfa meadow.

The term hibernation is used only in a general sense, as overwintering adults merely keep in hiding under cover during cold weather and are dormant only at temperatures below freezing. At air temperatures only a few degrees above freezing they have been observed hopping about actively on the frozen ground after the covering of grape leaves had been removed from about the base of the vines.

FEEDING OF ADULTS AND SPRING ACTIVITY.—As with *Erythroneura comes*, the three-banded leafhopper feeds on a variety of plants before the grape leaves appear in spring, but once it begins feeding on grape foliage it does not change until the leaves are destroyed by frost. Similarly the species has not been found breeding on any plant other than grape. The general movement to grape foliage in the spring, however, seems to take place somewhat later than with certain other species. On South Bass Island, Lake Erie, in 1921, new shoots and grape leaves in several vineyards were found to be severely injured by large numbers of overwintering adults of *E. vulnerata* var. *vulnerata* on May 18. The three-banded leafhopper (*E. tricincta* var. *cymbium*) was found to be present on the ground among fallen leaves in large numbers, but was not found abundant on the new growth until after June 10. After that date, it remained the predominant form until the close of the season. During 1922, nymphs of *E. comes* were recorded in vineyards considerably earlier than nymphs of *E. tricincta* var. *cymbium*.

INCREASE OF GRAPE LEAFHOPPERS

In most of the grape-growing districts from Lake Michigan eastward, there has been a very marked increase of grape leafhoppers in the last few years, this probably being the effect of several mild winters in succession. Vineyards in numerous localities in northern Ohio, which the writers' records show were practically uninfested in 1919, are now heavily infested. In several localities records of the infestation have been obtained each season since 1919. These records show that all species of grape leafhoppers have increased and that of the forms present *Erythroneura tricincta* var. *cymbium* has increased most rapidly.

CONTROL

Control experiments against several of the species of grape leafhoppers occurring in northern Ohio vineyard districts have given results not significantly different from those reported by previous workers. Spraying is not effective against adults, but must be directed against the nymphs. A nicotine spray of strength 1 to 1,200 thoroughly applied to the under surface of the grape leaves when the first-hatched nymphs have nearly completed their growth has been recommended. Such a spray will reduce infestation sufficiently to permit normal development and ripening of fruit and wood. A spreader in the spray is desirable. Of the substances tested, lime caseinate, ground glue, ground gelatine, saponine, potash, fish-oil soap, and resin fish-oil soap have given best results on grape foliage. More frequently, however, nicotine is applied in combination sprays used against other grape insects and diseases. In this case resin fish-oil soap proved best, the others not covering or adhering to the grape clusters so readily. Combination sprays were best applied by the trailer method, using special care to cover the under surface of the grape leaves. Because of the slowness and expense of application and the difficulty of timing the combination spray advantageously, the nicotine spray alone (with spreader), or when desirable with Bordeaux mixture, was applied with a spray boom of the type described by DeLong (*1*). Results were as satisfactory as the average results obtained by trailer spraying and the expense much less.

LITERATURE CITED

(1) DeLong, D. M.
1922. THE BOOM NOZZLE SYSTEM AND THE TRACTION DUSTER AS FACTORS IN GRAPE LEAFHOPPER CONTROL. *In* Jour. Econ. Ent., v. 15, p. 87–90, 1 pl.

(2) Fitch, Asa.
1851. CATALOGUE WITH REFERENCES AND DESCRIPTIONS OF THE INSECTS COLLECTED AND ARRANGED FOR THE STATE CABINET OF NATURAL HISTORY. *Reprint in* Lintner, J. A., Ninth report on the injurious and other insects of the State of New York, for the year 1892, p. 383–413 (original pages 47–69; *Erythroneura tricincta*, original description, p. 63), 1893.

(3) Hartzell, F. Z.
1912. THE GRAPE LEAFHOPPER AND ITS CONTROL. N. Y. State Agr. Exp. Sta. Bul. 344, p. 29–43, 3 fig., 4 pl.

(4) Johnson, Fred.
1914. THE GRAPE LEAFHOPPER IN THE LAKE ERIE VALLEY. U. S. Dept. Agr. Bul. 19, 47 p., 13 fig., 3 pl. Bibliography, p. 43–47.

(5) Lawson, P. B.
1920. THE CICADELLIDAE OF KANSAS. Kans. Univ. Sci. Bul., v. 12, no. 1 (Ent. no. 4), 376 p., 27 pl.

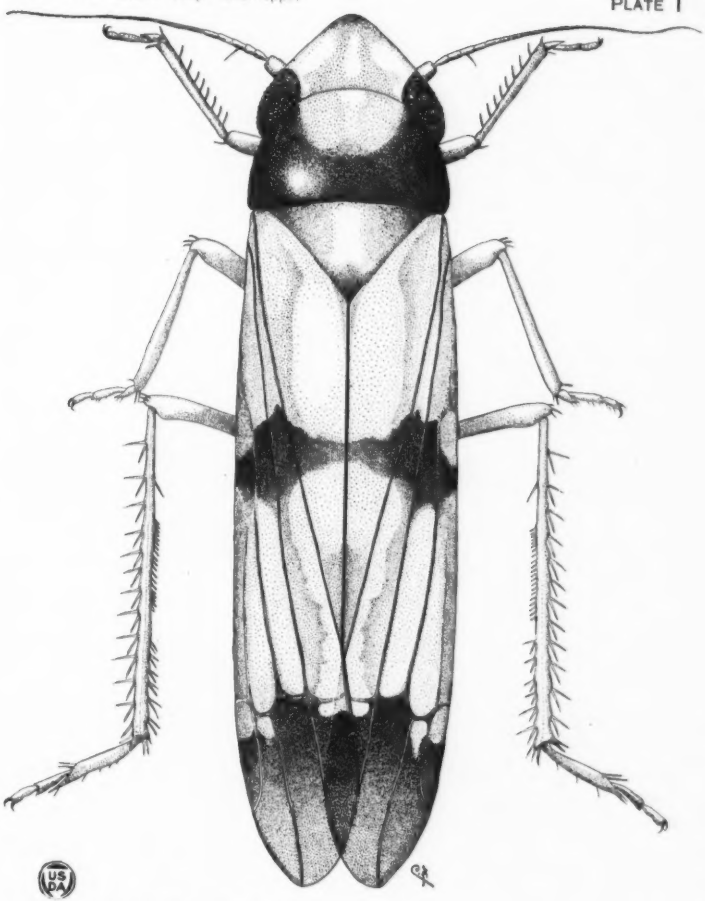
(6) McAtee, W. L.
1920. KEY TO THE NEARCTIC SPECIES AND VARIETIES OF ERYTHRONEURA. *In* Trans. Amer. Ent. Soc., v. 46, p. 267–322.

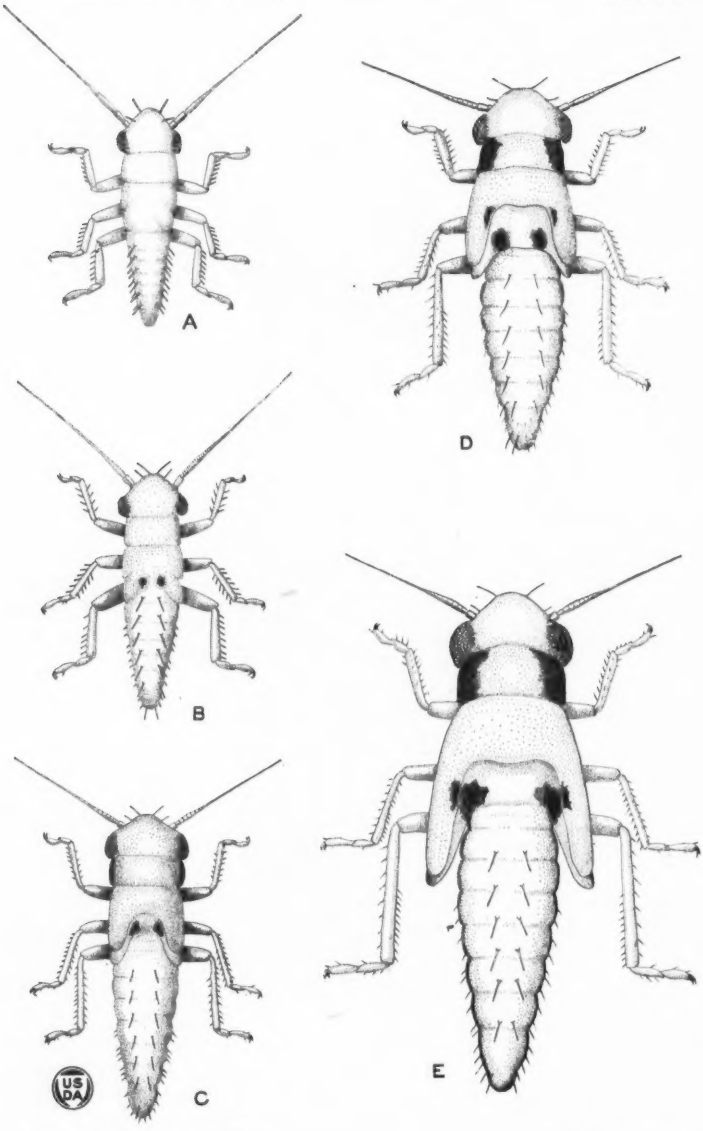
PLATE 1

Erythroneura tricincta var. *cymbium:* Adult.

The Three-banded Grape Leafhopper

PLATE I





A.
B.
C.
D.
E.

PLATE 2

Erythroneura tricincta var. *cymbium:*

A. First nymphal instar.
B. Second nymphal instar.
C. Third nymphal instar.
D. Fourth nymphal instar.
E. Fifth nymphal instar.

SOME MORPHOLOGICAL RESPONSES OF THE HOST TISSUE TO THE CROWNGALL ORGANISM [1]

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INTRODUCTION

In a previous paper (*1*) [3] the writer has described inoculation studies of crowngall on tomato stems in which it was observed that needle punctures immediately released liquid which flooded the neighboring intercellular spaces and formed water-soaked areas in the surrounding tissue. Liquid which was expressed from a tomato stem was found to exert a positively chemotactic influence upon the crowngall organism, *Bacterium tumefaciens* Smith and Town. When applied to the surface of the puncture, they entered and distributed themselves through the water-soaked area. The galls which developed in the mature tissue were found to coincide closely in outline with these water-soaked regions. When the water-soaked regions were extended by bruising, the bacteria migrated to the limit of the continuous channel of liquid, which was as far as eight centimeters in some cases. The bacteria were observed consistently *in situ* in an intercellular position, both in free-hand and in paraffin sections from five series of inoculations which were examined at two-day intervals from the time of inoculation until distinct hyperplasia, hypertrophy, and vascular elements developed.

The earlier paper made no attempt to describe the abnormal host tissue. Herewith is given a report, previously briefly noted (*2*, *3*), of the studies made upon the responses of the host tissue to the bacteria, including a description of such phenomena as "secondary tumors" and "tumor strands."

HISTOLOGICAL STUDIES OF CROWNGALL ON TOMATO

At the time when observations were made on the location of the bacteria, an opportunity was provided for studying the course of development of the gall. This was done in the following manner. In a preliminary series, five inoculations were made on each stem of two dozen tomato plants. A different plant was examined every day and notes were taken relative to changes that occurred during the formation of the galls. In three subsequent series the examination was made only every other day because the changes were too slow to show any marked difference in twenty-four hours. These four series varied somewhat in the speed with which certain developments appeared, but the order was practically the same. For the most part, examinations were made on free-hand sections mounted in lacto-phenol (equal parts of phenol,

[1] Received for publication April 16, 1923.

[2] The writer takes pleasure in expressing his indebtedness to Dr. L. R. Jones and Dr. G. W. Keitt, of the University of Wisconsin, and also to other members of the faculty, especially to Dr. E. J. Kraus, for many valuable suggestions in the prosecution of these studies.

[3] Reference is made by number (italic) to "Literature cited," p. 435.

lactic acid, glycerin, and water) to which a little eosin had been added. This type of preparation was employed because it was easier to handle, and it showed more clearly the location of the organism, its migration, and its action on the middle lamella than did paraffin sections. The latter were used in an additional series as a check upon the fresh preparations and for the purpose of making photomicrographs.

The developments in one of the two-day series are listed here in more or less detail. The examinations were made with the use of low power, high power, and oil immersion objectives together with a complete range of oculars. Polarized light was employed for examination of the walls, pectic granules, and crystals. The inoculations were made on March 10, 1922, on tomato plants grown in the greenhouse. Since the morphology of the normal tomato stem is so well known, no description of it is given here.

TWO-DAY-OLD INOCULATION

Bacteria were noticed in considerable numbers along the path of the needle and inside of ruptured cells. Many of the intercellular spaces, especially those above and below the puncture, in the pith and in the cortex were found to contain the organism. The walls bordering such spaces were tinged with yellow, as is frequently the case with wounded tissue. When very dilute gentian violet was added, this yellow portion took up the stain more readily than did the other regions. This increased affinity of the walls for stains is probably the explanation of the difficulty found in demonstrating the bacteria with the conventional methods. The invading organism seemed to have some influence on the walls, which resulted in more or less swelling and loss of the property of double refraction of light. Lateral migration of the organism for a short distance from the puncture had occurred in the subepidermal region (pl. 1, A), where there are comparatively large and frequent intercellular spaces. It is noteworthy in this connection that the first layer of cells beneath the epidermis frequently contains anthocyan and has a higher sugar content than some of the other tissues. Whether or not this has an influence on the bacteria has not been determined. In this region also the phellogen has its origin. So it is not surprising that a stimulus to cell division in this portion of the stem should find a ready response.

FOUR-DAY-OLD INOCULATION

To the naked eye a slight swelling was evident about the puncture. Under the microscope this was much more apparent. The cortical cells had swollen and a few cell divisions had occurred. The old walls had increased in thickness while the yellow regions surrounding the position of the bacteria were extended. The organisms were easily located in some of the intercellular spaces and were traced a short distance into the middle lamellae between the adjacent cells. In the pith little change had taken place except a browning of certain of the intercellular spaces about the bacteria and occasional divisions. It was noticed again that the walls bordering the bacteria took the stain more heavily. With polarized light it was again observed that the yellowed walls near the organisms were not doubly refractive. No real difficulty was experienced in differentiating the bacteria from the pectic granules in the middle lamella or from crystals. In cases of doubt the former were dissolved in

hydrochloric acid followed by potassium hydroxid, while the latter were readily distinguished with polarized light. In some instances bodies resembling bacteria were observed in the cut cells as reported by Smith (*5, p. 253*). A few could very easily have escaped from the intercellular spaces at the time the section was made. Little confusion was experienced with chondriosomes because the mounting fluid destroyed these bodies in a short time. The cells which had been ruptured by the inoculating needle and then invaded by bacteria appeared to be dead. The puncture was bordered by the discolored walls of such cells.

SIX-DAY-OLD INOCULATION

The new gall was easily seen with the unaided eye. Under the microscope the swelling was found to be due to the hypertrophy of the cortical cells rather than to much proliferation of tissue. Cell division was found to occur to the extent of several new cells in the subepidermal region surrounding the original points of bacterial invasion of the intercellular spaces. The proliferation about the intercellular spaces in the pith was increased by new walls, which were laid down in the older cells, facing the position occupied by the bacteria. The new walls did not pass through the middle of the cells but rather cut off smaller portions on the side nearest the organism. This phenomenon is still more striking in some of the eight- or ten-day-old inoculations (pl. 1, B). So it appears that both in the pith and in the cortex small hyperplastic cells are formed within the older cells. The formation of the small cells by division of the larger ones is reported by Smith (*8, p. 2*) ". . . the cells have not changed places but the change has occurred *in situ* by conversion of the large cells wholly normal into congeries of small cells having all the characteristics of tumor cells and visibly surrounded in many cases by the stretched wall of the original cell."

Observations were made similar to those already mentioned on the presence of the bacteria and the changes in the walls surrounding them. The cells which were originally invaded by the bacteria appeared collapsed against the side of the puncture. Their walls were brown.

EIGHT-DAY-OLD INOCULATION

Macroscopically, the gall was conspicuous. With the low power of the microscope the regions of hypertrophy and hyperplasia appeared enlarged. The small gall cells (pl. 1, D and E) formed a sheath (*f*) about the position of the bacteria (g). In this stage it appears that the arrangement of the hyperplastic cells points out the source of the stimulus. Outside this sheath in the cortex there was a considerable region of hypertrophy where the cells were less and less swollen until the normal tissue was reached. In the pith the response was not so great as in the cortex, but a distinct sheath of cells was observed. The region of hypertrophy was not conspicuous, if present at all. When the sheath of cells formed around the intercellular spaces that were invaded in a more or less straight line, they produced a cylindrical structure that appeared like a strand of tumor tissue extending away from the point of inoculation (pl. 2, A and B). The hyperplastic regions in this stage appeared to be collections of more or less irregular cylinders of this nature. It seems unlikely that this type of development might have been produced by appositional growth in one direction only (Smith *8, p. 34*). The

bacteria were again observed in the yellowed intercellular spaces and in no other position. One could trace their course through the tissue by the changes they produced in the properties of the adjacent walls. No evidence was found to indicate that the bacteria were inside of any of the living cells.

TEN-DAY-OLD INOCULATION

A general increase was noted of all the tissues previously mentioned. The affected tissue of the tomato had become so distorted that the intercellular spaces which were filled with gas showed a disorderly and bizarre arrangement. Calcium oxalate crystals were observed in unusual quantities in some of the larger cells. A certain amount of distortion among the large cells indicated that pressure was being exerted by the gall tissues. Up to this time the xylem showed no material increase in thickness. The bacteria were found as usual in the intercellular spaces in association with some of the yellowed walls.

TWELVE-DAY-OLD INOCULATION

There was a continued enlargement of the gall area and the individual tissues of the tumor. The xylem and phloem bordering the gall tissue showed an increased width. This was possibly due to an unusual amount of cambial activity under the influence of the stimulus provided by the bacteria. By this time the hyperplastic regions had encroached upon the hypertrophic areas to such an extent that the earlier structure of a collection of cylindrical gall tissue about invaded intercellular spaces was partly obscured.

FOURTEEN-DAY-OLD INOCULATION

No new structures were noticed, but a further enlargement had taken place in all parts. By this time the gall had developed the regions of thin-walled rapidly dividing cells intermingled with those of large hypertrophic cells which are characteristic of old galls. The former appeared to contain no air spaces, while the latter had large irregular ones. The bacteria were still to be found in some of the yellow walled intercellular pockets. These were more widely scattered than they were in the younger galls and appeared not to contain so many bacteria.

SIXTEEN-DAY-OLD INOCULATION

Growth had continued in all parts. For the first time, some of the gall tissue which originated in the subepidermal region showed differentiation into vascular elements. There was no connection between this tissue and any of the tissue produced by the cambium, so the tracheids must have been produced by tumor cells which in turn came from cortical cells. Smith (*5, p. 248*) has reported similar findings. The groups of calcium oxalate crystals were increasingly numerous.

EIGHTEEN-DAY-OLD INOCULATION

Vascular tissue was noted in larger quantities in the portion of the gall derived from the cortex. It was found to appear also in gall tissue which originated in the pith. The bacteria, although still present in

large numbers, were not so easy to locate as in the younger galls. Considerable increase was noticed in the normal xylem near the gall area. It appeared that the xylem near the puncture was several times broader at this point than it was in a normal region on the same stem. Similar observations were reported by Smith (*8, p. 2*).

TWENTY-DAY-OLD INOCULATION

By this time the young gall had developed until it had the characteristics of a mature tumor. Vascular tissue appeared abundantly in the gall tissue, no matter what its original source. The earlier structure of a group of cylindrical strands surrounding the position of the bacteria was entirely obscured by the confusion of intermingled types of gall tissue. The growth of the different tumor tissues had separated the original centers of infection until here, as in still older galls, they were comparatively inconspicuous.

A comparison of these observations was made with others on tomato stems which were punctured but not inoculated. It appeared that only a very small part of the swelling was a response to the wound. This has never been found to exceed that which appeared on the four-day-old inoculation. No yellowing of the walls had developed around the intercellular spaces that were flooded by liquid, either in the subepidermal region or in the pith. However, a yellowing was noticed in the dead cell walls and, on either side of the puncture, in the region of the endodermis. In the latter case the color persisted for a number of days, but finally disappeared. The nature of this colored substance was not determined. A few cell divisions were found to occur next to the puncture so that the cavity in the pith was completely filled up. In some cases, after two weeks' time, the hole through the cortex became partially filled.

The stimulus for the development of tumor tissue seems to be some product of the bacteria (Smith *6, p. 184*). This appears to diffuse more or less equally in all directions through the surrounding tissue. When the stimulus is strong there seems to be a tendency toward the formation of hyperplasia; when it is weak a hypertrophy results. However, continued action of the weaker stimulus may induce the enlarged cells to divide up into smaller ones. When the stimulus is very weak it tends to serve merely to stimulate the normal processes of the plant to greater activity. This may be seen in the unusual development of xylem at the edge of the gall. It seems quite plausible as Smith has suggested (*7, p. 179*) that after the gall is once started, the disturbed metabolism of the gall tissue may furnish a sufficient stimulus to incite the continued development of tumor cells.

As yet the writer has made no intensive study of nuclear phenomena in the activities of the cells immediately adjacent to the intercellular spaces containing bacteria. However, it was noted, especially in the pith, that the nuclei seemed to divide most frequently in the portion of the cell that was nearest the bacteria (pl. 1, C). What the nature of this attracting influence may be, or the means by which it attracts the nuclei, is not understood. However, this phenomenon appears to be intimately associated with the loss of polarity in the cells and the consequent development of the tumor tissue. It appears that the stimulus from the bacteria attracts the nuclei to the adjacent portion of the cell, and then influences them to divide. The result is that the new wall is formed

in that portion of the mother cell nearest the bacteria. Thus a small daughter cell is interposed between the bacteria and the remainder of the mother cell. This may be seen in Plate 1, B. So it appears that the new tumor cells form a sheath about the bacteria and indicate the source of the stimulus. In the tomato this condition is most evident in galls eight or ten days after inoculation. In the later stages, in many cases, such a confusion of proliferating tissue appeared that the original relationships were lost.

Whether or not the bacteria were able to migrate through the tissue, beyond the intercellular spaces which were water-soaked at the time of infection, has not been definitely determined. The writer has never found any evidence to indicate that the bacteria might pass through the tissue in any manner other than through the intercellular spaces. It appears that they can influence the cell walls a short distance from the intercellular space so as to produce the yellowing previously mentioned. However, there is every reason to believe that the extent of the original invasion might be enlarged by any influence, such as injury or sudden change of temperature, which would cause liquid to occur in the intercellular spaces. It also appears that if the area of original invasion were one that was rapidly elongating, then this area might be stretched out over a considerable distance and the infection would then appear as a long-drawn-out tumor or "tumor strand." This type of gall may appear when an inoculation is made into the region of elongation near a condensed bud such as one finds in sunflower, sweet pea, Paris daisy, and tobacco. The elongated tumor is sometimes manifested as a "primary gall" with one or more "secondary galls" and "tumor strands."

DEVELOPMENT OF "TUMOR STRANDS" AND "SECONDARY TUMORS"

"Tumor strands" and "secondary tumors" have not been easy to produce. Several thousand inoculations have been made on tomato, raspberry, blackberry, tobacco, sunflower, Paris daisy, and sweet pea on parts other than the rapidly elongating regions. These produced galls readily but never produced any "secondary tumors." These phenomena never have been secured except in cases where the puncture was made close behind the growing point of a condensed bud such as occurs in sunflower, tobacco, sweet pea, and Paris daisy, but not in tomato. Even in these instances the number of "secondary tumors" secured was seldom above 5 per cent of the number of inoculations. The most favorable position for making inoculations to secure "secondary tumors" is described by Smith (*4, p. 23*), "At the time of inoculation the stems were soft and rapidly elongating, and the needle pricks were made in what was then the top of the plant." But so far as the writer has been able to ascertain, none of the previous workers on crowngall have taken into account the influence of either (1) the water-soaking of the intercellular spaces or (2) the elongation of the condensed bud[4] in the production of "secondary tumors."

On February 7, 1922, a number of inoculations were made with a fine needle in the rapidly elongating buds of some Spencer sweet peas grown in the greenhouse. On about the fifth day thereafter, distinct swellings

[4] After this manuscript was submitted to the publisher an article was received by ROBINSON, W., and WALKDEN, H. A CRITICAL STUDY OF CROWN GALL. *In* Ann. Bot., v. 37, p. 299–324, 4 figs., pl. 5–6. 1923. Literature cited, p. 322–323. It was noted with satisfaction that in this publication conclusions very similar to our own upon the relation of the elongation of the growing tip to the development of "tumor strands" and related points were reached independently by these authors.

were noticed on some of the main stems at the points of inoculation. In about 12 days the first signs of "secondary tumors" began to appear. After about four weeks it was noted that only about 5 per cent of the stems showed any "secondary tumors" at all, and of this number great variation in the infection was manifest. This may be seen in Plate 3, which shows the galls about seven weeks after inoculation. In each case the needle passed through the leaves marked a and struck the stem proper at b, where the "primary gall" developed. "Secondary galls" appeared at intervals above and below. It is to be noted that the stems A and E received such a heavy inoculation that the bud was unable to expand, and consequently we have the stem ending in a mass of gall tissue. C and D were less completely involved and were able to expand to a certain extent. In each case three nodes developed galls above the point where the needle struck the stem. In the case of B the stem was still less involved, so that "secondary galls" appeared only along the internodes above and below the point where the stem was inoculated. Here there is some prevention of expansion, but it is not nearly so great as in any of the other cases.

Serial sections were cut through the "secondary galls" marked c and d. Plate 4, A, shows a section cut between b and c. The positions are marked e where the walls were stained more heavily as a result of the presence of the bacteria. In the center at st a small amount of proliferation appears. Here we have the early stages of "tumor strand" formation. Figure B shows in section the gall c of Plate 3, B. The position marked X on the section was directly above the strands. So far, in this gall there has been no differentiation into vascular tissue. Plate 4, C, shows the "tumor strand" as it proceeds on to the next gall. There was no connection in all its course between this "strand" and the vascular tissue. The "strand" opens out into the gall marked d of Plate 3, B. Thus we find interesting developments similar to those that Smith (*5, p. 236*) has described in the daisy. Plate 4, D, shows a section of this gall. It seemed that a stimulus similar to that which operated in the tomato stem (*p. 428*) to produce vascular tissue out of tumor cells had acted here. This development appeared to start in one place and to spread in such a way as to produce a more or less spherical mass of woody tissue. No evidence was found to indicate that there was any connection between the type of tissue inoculated and the type of tissue produced by the "secondary tumor." This does not seem to conform to Smith's statement (*5, p. 245*) that, ". . . the structure of the secondary tumor repeats that of the primary tumor . . . ." If such were the case one would expect that two galls resulting from the same "tumor strand" as c and d of Plate 3, B, would have the same structure. But we have seen that one is vascular in a large degree, while the other is entirely parenchymatous. Smith employs the point given above as an argument for the invasive character of the "tumor strand" and "secondary gall" (*5, p. 245*), but he does not apply it without qualification, either to teratomatous tissue (*5, p. 247*) or to woody tissue (*5, p. 248*).

Results similar to those obtained in experiments on the sweet pea have also been secured on the sunflower. During the last week in July, 1922, 150 inoculations were made on sunflowers grown in the experimental garden at Madison, Wis. At the time of inoculation the plants stood about 2 feet high. In each case only one puncture was made on a plant, and that passed through the rapidly elongating region of the condensed bud. The plants were harvested on October 12, 1922. At this time they stood 8 to 11 feet high and bore large heads from which the seeds were

shattering. The points on the stems where the "primary galls" appeared had been raised to about 5 feet from the ground. Although a large number of these "primary galls" showed evidence of elongation, good "secondary galls" were secured on only about 6 per cent of the plants. These appeared sometimes on the leaf petioles and sometimes in the pith.

The influences of heavier or lighter infection in the rapidly elongating region of sunflower, similar to those noted in sweet pea, may be seen in Plate 5, A and B. In A the infection involved 15 internodes and prevented their normal expansion, so that the galls remained in a more or less compact group. On the contrary, in B the infection was not so heavy, and expansion was obstructed in only the lower ones of all the internodes involved. Here are found "secondary galls" c and d appearing on the petiole of a leaf which is removed from the "primary gall" b by 13 internodes and a distance of 49 cm. Sections cut through the petiole one-half cm. below d showed a "tumor strand" directly in line with the "secondary gall." This strand consisted of cortical tissue which had been modified by the presence of the bacteria so that the walls have a different staining reaction. Limited regions of hypertrophy and still more limited areas of hyperplasia were found.

"Tumor strands" were also found in the pith. In Plate 5, C, the dark mass of gall tissue which arose about the needle puncture at e may be seen. From this a line of tumor tissue extends upward over 60 cm. At f the tumor tissue occupied almost the exact center of the pith and measured about 3 mm. across. It seemed to be moist and living, while the surrounding pith cells appeared dry, white, and dead. Paraffin sections were cut from the tumor tissue in this position. It proved to be composed of a series of "tumor strands" that showed various degrees of change. In Plate 5, D, some small tumor cells (g) may be seen surrounded by large tumor cells (h) which have a characteristic staining reaction, while pith cells that have been collapsed by the pressure of this and other strands may be seen at i. In figures E and F of the same plate a larger group of small tumor cells may be seen at g. Here vascular elements have developed at j.

Inoculation experiments on tobacco, though limited to 50 plants, produced rather similar results. In most cases, as was noted in the sunflower, there was considerable elongation of the primary lesion. In a small percentage of the inoculations "secondary tumors" were secured which involved several internodes. In one case five leaves, separated by eleven internodes, were involved as a result of a single puncture inoculation. Two of the leaves were so completely invaded that they appeared as irregular masses of gall tissue. The tip of one of these heavily involved leaves was separated by 9 cm. from the puncture.

Similar experiments on the Paris daisy for one reason or another have failed. However, such phenomena have been very completely described on this plant by Smith (*4*). The writer is of the opinion that these phenomena on the Paris daisy are similar to those on the sweet pea, sunflower, and tobacco.

HISTOLOGICAL STUDIES OF REGIONS OF ELONGATION

Inasmuch as "secondary galls" have never been secured except when the inoculations were made into rapidly elongating regions close behind condensed buds, it seemed essential for the understanding of the method by which these structures are formed to study the morphology of the

region inoculated. Consequently, a series of sections were cut both longitudinally and transversely through the growing tips of the sweet pea, sunflower, and Paris daisy.

A median longitudinal section through the sunflower may be seen in Plate 6, A. Intercellular spaces have been located in the pith as far above the usual position of the puncture (base of arrow) as the distance indicated by the arrow. The great condensation of the internodes is shown by the leaves and leaf primordia on the sides. Those shown are, of course, by no means the total number. It is estimated that only about one out of four of the total number of young leaves appears on one side of the section. Thus it may be seen that the area covered by the arrow extends over sixteen internodes. Of course, it must be borne in mind that the buds of individual plants vary somewhat.

The cross section of the tip of a sunflower stem shown in Plate 6, B, was taken 0.13 mm. below the apical cell of the growing tip at the place indicated by the transverse line in A. Here it may be noted that the intercellular spaces are already formed in a few places in the pith (pl. 6, D) and are comparatively well developed in the leaves that are differentiated. Well formed though small intercellular spaces have been observed even in a portion of the smallest leaf section (pl. 6, C). Since three of the older leaves which have their origin above the point of puncture do not show in B, it is quite evident that the intercellular spaces occur in more than seventeen internodes above the position of the puncture.

So it appears that the liquid released by a puncture in the rapidly elongating region of the sunflower bud could find a channel in the intercellular spaces of both cortex and pith to spread over the distance occupied by more than fifteen internodes. At the same time, the linear distance of migration of the organism at the time of inoculation to cover this number of expanded internodes, as may be seen in Plate 6, need not be more than seven mm. It seems unquestionable that the bacteria, once inside such a wound, would migrate to the limits of the avenue provided by the flooded intercellular spaces. Thus "secondary galls" separated by a number of internodes from the puncture might be produced at any position or series of positions where conditions were favorable. The actual distance of separation would depend upon the amount of expansion as the water-soaked region elongated.

It is not surprising then that "secondary galls" and "tumor strands" occur as far as 60 cm. away from the point where the needle entered. Rather it is more surprising that "secondary galls" are not produced in a higher percentage of trials. In actual practice it appears that the water soaking does not progress even as far as the thirteenth node except in 1 or 2 per cent of the inoculations in the region of elongation.

At the same time, it is evident that "secondary galls" and "tumor strands" are not really secondary at all from the standpoint of invasion. They are provided with bacteria from the same inoculation that produced the "primary gall." Their appearance of being secondary is the result of their separation from the point of entry by the processes of elongation, and also of the presence of a much smaller number of bacteria in a region that is less completely occupied by liquid. This accounts for their appearance later than the "primary gall" and their being smaller in size.

A similar situation has been found as the result of examinations made of longitudinal and cross sections of Paris daisy and sweet pea. Photomicrographs of median longitudinal sections through the growing tips of

these plants may be seen in Plate 6, E and F. Cross sections through the Paris daisy showed intercellular spaces seventeen internodes above the point punctured in the leaf primordia and fifteen internodes in the pith. In the sweet pea intercellular spaces were found in the pith seven internodes above the point of puncture.

SUMMARY

1. The crowngall bacteria have been observed consistently in an intercellular position in five series of inoculations on tomato stems which were examined at two-day intervals until the galls showed mature characters, that is, distinct regions of hyperplasia, hypertrophy, and vascular elements.

2. The bacteria were found to be associated with a change in neighboring cell walls which was manifested by a change in color, in staining reactions, and in the power of double refraction of light.

3. The bacterial stimulus appears to attract the dividing nuclei to the adjacent part of the cell and thus to disturb the polarity of the cell.

4. The walls resulting from the early divisions were laid down in the portion of the mother cell near the intercellular space containing the bacteria.

5. The divisions of the cells in the early stages of gall formation were observed to form a more or less distinct sheath about the position of the bacteria.

6. The manner of cell division in the early stages indicated the source of the stimulus and the position of the bacteria. In older galls this relation was not so clear.

7. The younger galls appeared to be composed of groups of sheaths or strands of tumor tissue surrounding invaded intercellular spaces.

8. Calcium oxalate was found deposited in much larger quantities in gall tissue than in normal tissue.

9. "Tumor strands" and "secondary tumors" have been secured only when the inoculation puncture was made in the region of elongation close behind the condensed buds of such plants as sunflower, sweet pea, and tobacco. They were secured in only about 5 per cent of the inoculations.

10. Intercellular spaces have been found in the condensed buds of sunflower to extend from the usual position of puncture up past fifteen nodes. The internodes in these buds were observed to be very greatly condensed. A similar situation has been found in sweet pea, tobacco, and Paris daisy. So in all these plants, it appears that the liquid released by a puncture might flood the intercellular spaces for a number of internodes, and the bacteria, migrating through the liquid might involve the same amount of tissue.

11. The elongation of the internodes in the condensed bud above the puncture was found to be of very great importance in the separation of the "secondary galls" from the point of puncture.

12. A heavy inoculation of the growing region prevented the expansion of the internodes.

13. No evidence has been found to show that "secondary tumors" and "tumor strands" invade the normal tissues. They appear to be part of the "primary gall" which was carried up by elongation of the invaded region.

14. The specialized tumor tissue found in "secondary galls" appeared to develop independent of any relation to the "primary galls."

LITERATURE CITED

(1) RIKER, A. J.
1923. SOME RELATIONS OF THE CROWN-GALL ORGANISM TO ITS HOST TISSUE. *In* Jour. Agr. Research, v. 25, p. 119–132, 5 pl. Literature cited, p. 132.
(2) ———
1923. THE LOCATION OF THE CROWN-GALL ORGANISM IN ITS HOST TISSUES. *In* Phytopathology, v. 13, p. 43.
(3) ———
1923. SOME MORPHOLOGICAL RESPONSES OF THE HOST TISSUES TO THE CROWN-GALL ORGANISM. (Abstract.) *In* Phytopathology, v. 13, p. 43.
(4) SMITH, Erwin F.
1912. THE STRUCTURE AND DEVELOPMENT OF CROWN-GALL: A PLANT CANCER. U. S. Dept. Agr. Bur. Plant Indus. Bul. 255, 60 p., 2 fig. 109 pl.
(5) ———
1916. STUDIES ON THE CROWN-GALL OF PLANTS. ITS RELATION TO HUMAN CANCER. *In* Jour. Cancer Research, v. 1, p. 231–309, 25 pl. Bibliography, p. 258.
(6) ———
1917. MECHANISM OF TUMOR GROWTH IN CROWN-GALL. *In* Jour. Agr. Research, v. 8, p. 165–188, pl. 4–65. Literature cited, p. 185–186.
(7) ———
1920. PRODUCTION OF TUMORS IN THE ABSENCE OF PARASITES. *In* Arch. Dermatology and Syphilology, v. 2, p. 176–180.
(8) ———
1922. APPOSITIONAL GROWTH IN CROWN-GALL TUMORS AND IN CANCERS. *In* Jour. Cancer Research, v. 7, p. 1–105, 4 fig., 28 pl. References, p. 47–49.

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PLATE 1

Photomicrographs of cross sections of tomato-stem galls showing some responses of the cells which surround the bacteria.

A.—A cross section of a two-day-old inoculation showing the puncture, which is lined with bacteria at a, and lateral migration of the bacteria in the subepidermal region at b. ×56.

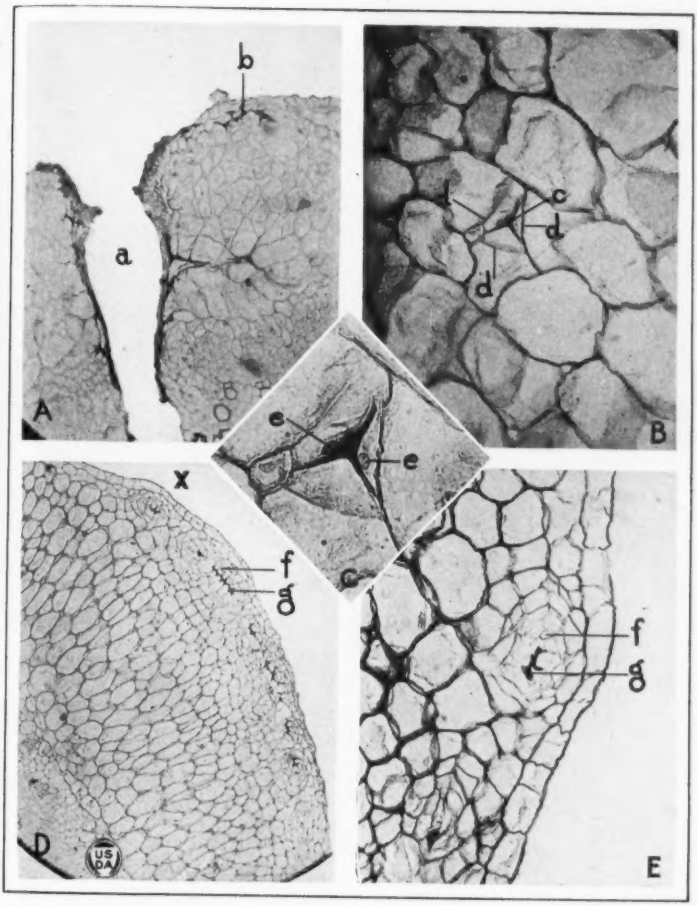
B.—A cross section of pith from an eight-day-old inoculation showing an intercellular space at c which contains bacteria. New walls (d) have been laid down in the surrounding pith cells in the portion of the cell nearest to the position of the bacteria. ×223.

C.—An enlargement of the invaded intercellular space shown in B. Here it may be noted that the nuclei e, lie in a position close to the bacteria, as if in response to some stimulus. This dislocation of the nuclei may assist in explaining the loss of polarity in gall cells. ×502.

D.—A cross section in the cortex of a tomato stem eight days after inoculation. Small gall cells are found to form a sheath, as at f, about the position of the bacteria, as at g. Here it appears that the host cells form a sheath about the position of the bacteria. ×56.

E.—An enlargement of the region at X in D. The hyperplastic cells (f) appear to form a sheath about the invaded intercellular space (g). ×169.

The preparations photographed for this plate were all paraffin sections stained with carbol fuchsin diluted 1:100, liquid measure, with water, and counterstained with light green in clove oil.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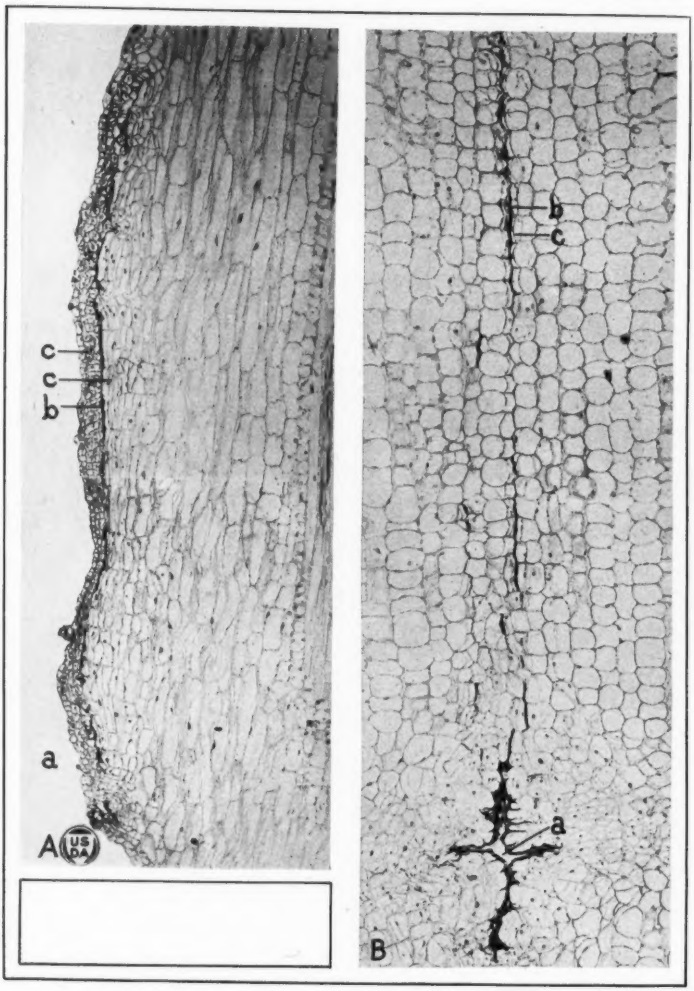
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PLATE 2

Photomicrographs of longitudinal sections of tomato-stem galls eight days after inoculation.

A.—A longitudinal section through the cortex of a gall similar to that shown in Plate 1, D. The puncture entered the stem at an elevation corresponding to a, but at a different level from this section. The invaded intercellular spaces (b) are surrounded by hyperplastic cells (c). ×59.

B.—A longitudinal section through the pith of a gall similar to that shown in Plate 1, B. The puncture was made at a. An invaded intercellular space may be seen (b) about which a few hyperplastic cells may be observed (c). ×59.

In both A and B it appears that the hyperplastic cells form a more or less cylindrical sheath about the invaded intercellular space which gives the appearance of a strand of tissue.

The preparations photomicrographed in this plate were made in a manner similar to those of Plate 1.

PLATE 3

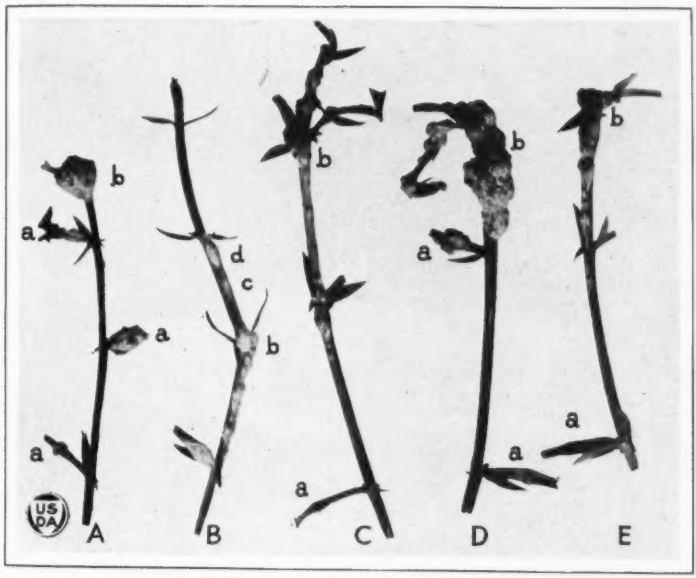
Crowngall on sweet peas which resulted from inoculation in the condensed region of elongation.

In each case the needle passed through the leaves marked a and struck the stem proper at b where "primary galls" developed. "Secondary galls" may be seen both above and below this position. Photographed about seven weeks after inoculation. $\times \frac{1}{10}$.

A and E.—The inoculation was sufficiently heavy to prevent the bud from expanding.

C and D.—The region of elongation was partly inhibited from expanding. In both cases three nodes above the puncture have developed "secondary galls."

B.—The elongation of the stem was still less inhibited. Serial sections were cut through this stem from b through the "secondary gall" d. Photomicrographs taken from these sections appear in Plate 4.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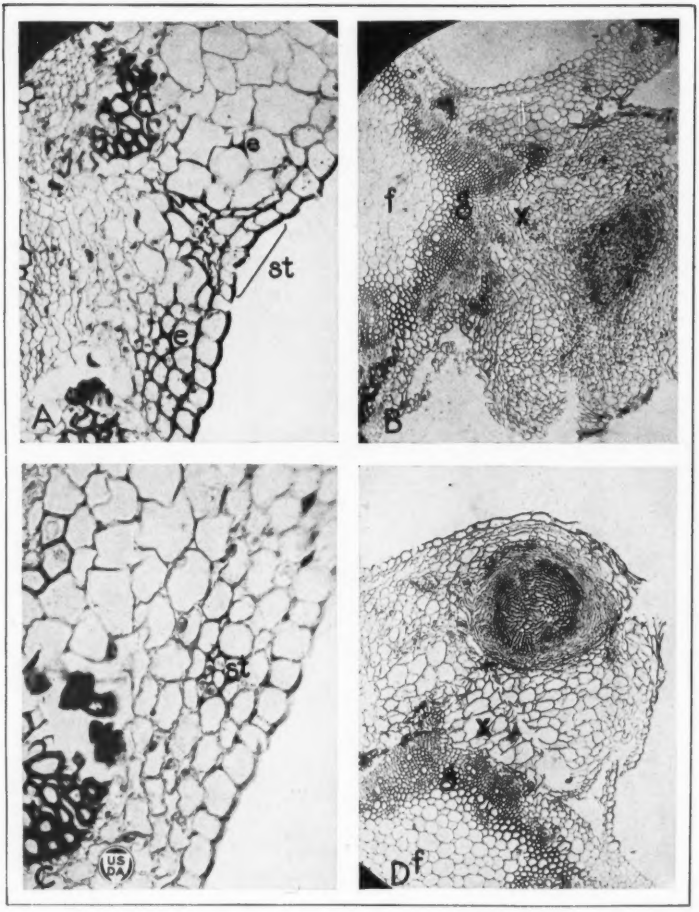

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PLATE 4

Photomicrographs showing cross sections of "tumor strands" and "secondary tumors" taken from Plate 3, B.

A.—A "tumor strand," st, which appeared in sections taken between b and c of Plate 3, B. It may be seen that some of the walls around certain intercellular spaces have taken the stain much more heavily, e, but that no proliferation has occurred about them. ×224.

B.—A section through the "secondary gall," c, of Plate 3, B. The position marked X is directly above and in line with the strand shown in A of this plate. A portion of the pith, f, and of the vascular tissue, g, of the stem may be seen at the left. ×56.

C.—A "tumor strand," st, which appeared in sections cut between c and d of Plate 3, B. Here also the walls have a changed staining reaction, and a few cell divisions have taken place.

This strand is directly above and in line with the position marked X on B of this plate. ×224.

It may be noted that the "tumor strands" and "secondary tumors" so far examined have had no connection with any vascular tissue.

D.—A section through the "secondary gall," d, of Plate 3, B. The position marked X is still directly above and in line with the strand shown in C of this plate. A portion of the pith, f, and of the vascular tissue, g, of the stem may be seen at the left. In the gall proper vascular tissue has developed in a more or less spheroid mass. It has no connection with any of the vascular elements of the stem proper. ×56.

PLATE 5

Crowngalls on sunflowers which resulted from inoculations in the condensed region of elongation.

A.—The needle passed through the position marked a. Fifteen internodes were involved so heavily that normal expansion was prevented. $\times\frac{3}{5}$.

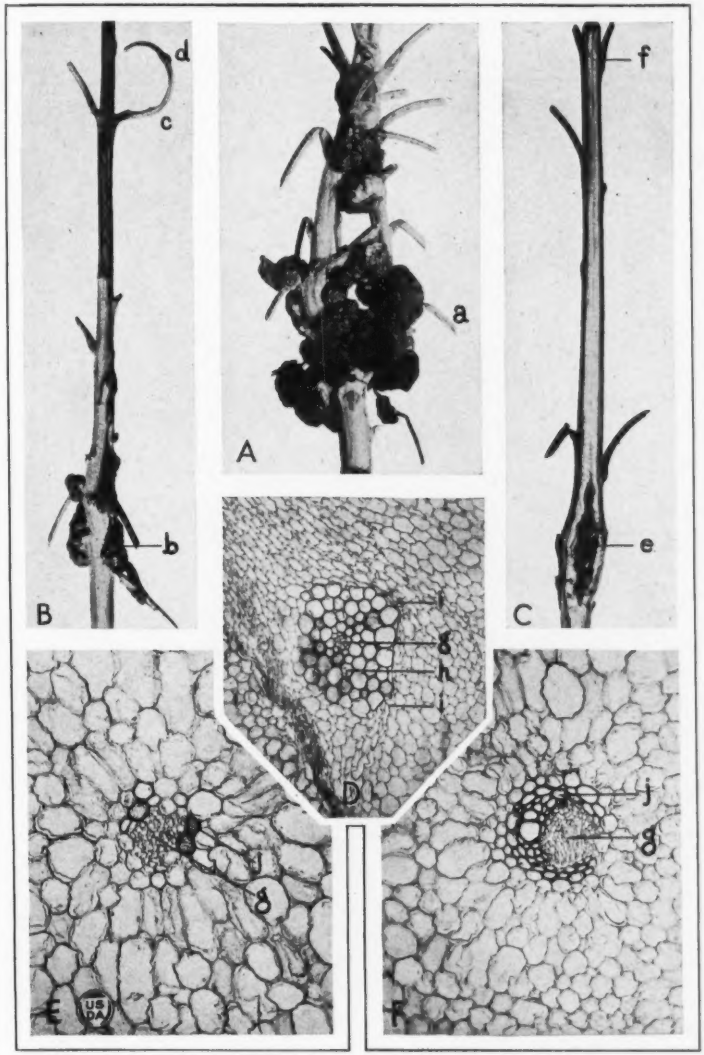
B.—The needle passed through the position marked b. Expansion was reduced only in the lower internodes of the thirteen involved. "Secondary galls" appear on a petiole at c and d. $\times\frac{1}{4}$.

C.—Similar to B except that the stem is split longitudinally revealing a dark line of tumor tissue which extends up from the region of puncture in the pith. Paraffin sections made from the pith at f showed this dark line to be a series of "tumor strands" which have developed in varying degrees. Some of them are shown in D to F. $\times\frac{1}{4}$.

D.—One of the strands of tumor tissue which appeared in a cross section of the sunflower pith taken from C, f. Hyperplastic cells may be seen at g, hypertrophic cells at h, and crushed pith cells at i. $\times 57$.

E and F.—Similar to D. Hyperplastic cells appear at g, while vascular elements have developed at j. $\times 57$.

The photomicrographs shown in this plate were made from paraffin sections stained with Flemming's triple stain.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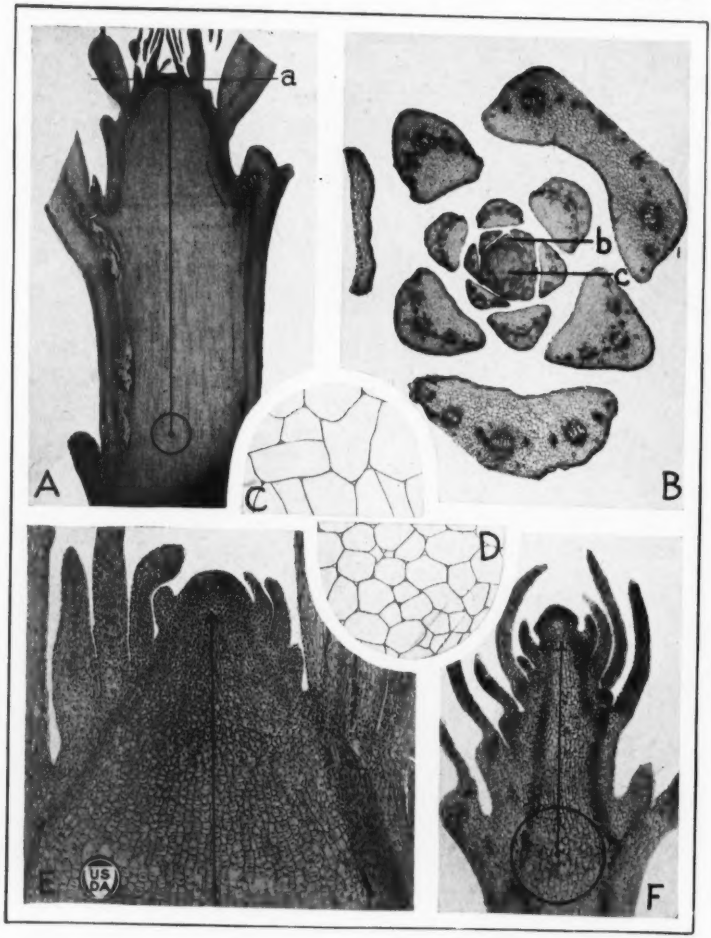
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PLATE 6

Histological studies of the regions of elongation in sunflower, Paris daisy, and sweet pea showing how inoculations in these regions might involve a number of internodes.

A.—A longitudinal section of the region of elongation of a sunflower. An inoculation puncture in this region frequently penetrates the area indicated by the circle at the base of the arrow which represents the diameter of the needle used. Intercellular spaces have been found extending upward throughout the distance covered by the arrow in the pith, and even further in the cortex. This distance is occupied by fifteen to twenty internodes. A cross section of the tip made at a position corresponding to the line a is shown in B. ×8.

B.—A cross section made through the region of elongation of a sunflower at a position 0.13 mm. behind the apical cell corresponding to the line a in A. Three of the outermost leaves have been removed. Intercellular spaces were found in the regions marked b and c. ×8.

C.—Camera lucida drawing showing the intercellular spaces in the leaf primordia in the position marked b in B. ×512.

D.—Camera lucida drawing showing the intercellular spaces in the pith in the position marked c in B. ×512.

E.—Similar to A, except made through the region of elongation of a Paris daisy. A cross section of this region revealed a situation similar to that found in the sunflower as shown in B, in regard to condensation of the internodes and the occurrence of intercellular spaces. At 0.14 mm. from the apical cell intercellular spaces were found in the pith. ×48.

F.—Similar to A, except made through the region of elongation of a sweet pea. In this case there are not so many internodes in the region of elongation as in the sunflower and Paris daisy. The circle at the base of the arrow indicates the relative size of the needle used. Intercellular spaces were found at 0.18 mm. from the apical cell. ×32.

The photomicrographs shown in this plate were made from paraffin sections colored with Flemming's triple stain. In each case the hairs about the young leaves have been blocked out.

THE MINIMUM MILK REQUIREMENT FOR CALF RAISING[1]

By A. C. RAGSDALE, *Chairman, Department of Dairy Husbandry,* and C. W. TURNER, *Instructor in Dairy Husbandry, Missouri Agricultural Experiment Station*

The problem of raising dairy calves on farms where the whole milk is sold to be utilized in the manufacture of dairy products or for consumption in the cities is one of ever-increasing importance. On these farms skim milk is not generally available and whole milk is so valuable that dairymen quite generally question the profitableness of raising dairy calves under such conditions. Under these circumstances a substitute for milk in raising calves would be a boon to dairymen, since it would greatly simplify the problem and undoubtedly be the means of saving many well-bred calves. A decreased cost of calf raising would also result from the sale of more of the milk now fed to calves.

Progress has been made in preparing calf meals that will gradually take the place of milk after the calf is 3 or 4 weeks old. However, complete success has not yet been attained. Another line of experimental work has for its object the determination of the earliest age at which calves can be weaned of milk and make satisfactory growth on grain and hay.

Fraser and Brand [2] of the Illinois station were the first to work on this problem. In a preliminary study, calves were cut off from milk at 42, 45, and 46 days of age. It was found that at these ages the digestive system had not developed sufficiently to utilize grain and hay. In a later trial the ration of skim milk was continued until 56 days, when grain and hay were again substituted. They fed on the average 152 pounds of whole milk and 435 pounds of skim milk. Better results were thus obtained and they concluded that it was possible to raise calves in this way on a moderate quantity of milk.

In studies at this station to determine the earliest age at which calves could be weaned and fed on grain and hay without causing serious digestive disorders, Fohrman [3] weaned two lots of three calves each at about 60 days of age and fed them hay and grain. The hay used was alfalfa and the grain mixture was composed of ground corn, 40 parts, wheat bran, 10 parts, and linseed meal, 10 parts. In the second mixture one-half of the linseed meal was replaced by blood meal.

Lot I gained 97.8 per cent of the normal increase in weight for that age and 91.9 per cent of the normal increase in height at withers. Lot II gained 96.7 per cent of the normal increase in weight for that age and 98.4 per cent of the normal increase in height at withers. Four of the six calves used in the experiment were males. Since the figures for the normal growth of females is undoubtedly lower than for the growth of males, the apparent per cent of normality would thus be increased for bull calves.

[1] Accepted for publication Oct. 3, 1923.

[2] FRASER, W. J., and BRAND, R. E. MILK REQUIRED TO RAISE A DAIRY CALF. Ill. Agr. Exp. Sta. Bul. 164, p. 437-458, 6 fig. 1913.

[3] FOHRMAN, M. H. MINIMUM MILK FOR CALF RAISING. Thesis for A. M. degree, University of Missouri, 1919.

After these preliminary studies it was deemed worth while to determine the feasibility of the general plan by securing additional data and by trying out other grain mixtures and roughages. As in the previous experiment, the calves were weaned at about 60 days of age and continued on the dry feed until 6 months of age. High-grade or pure-bred heifer calves were used exclusively. The calves were obtained as soon after birth as possible so that they could be kept growing at a normal rate. An effort was made to keep them in a thrifty, vigorous condition and as near normal in size and weight as possible. No records were kept of the feed consumed during this time, the plan being to give them good average herd care. The calves were fed whole milk until 3 weeks of age and then gradually changed to skim milk. At 10 days of age grain and hay were offered and the calves were encouraged to eat, so that they would become accustomed to the feed during the preliminary period.

Both the hay and grain were weighed to each individual calf and any portion rejected was weighed back. Part of the time cut alfalfa hay was fed, but the calves seemed to prefer the uncut hay. Due to the coarse stems of some of the soybean hay, it was found desirable not to cut the latter, so that the calves could select the choicer portions of the plant. No effort was made to limit the amount of feed, except that a maximum of 5 pounds per day of grain was established until the hay consumption reached 5 pounds per day.

In selecting the grain mixtures the chief point considered was to adopt one simple enough to be practical on the average farm and yet one which would contain all the essential factors in a balanced ration. The nutritive ratio of milk was taken as a guide.

Lot I was fed a grain mixture composed of ground corn 40 parts, wheat bran 10 parts, and linseed meal 10 parts by weight. The roughage fed was soybean hay.

Lot II was fed a grain mixture composed of ground corn 40 parts, wheat bran 10 parts, and ground soybeans 10 parts by weight. The roughage fed was alfalfa hay.

The calves were weighed three days in succession when put on experiment and thereafter at 10-day intervals until the close of the experiment. Each 30 days the average of three successive days was used. The height at withers was taken as the index of skeletal growth. These measurements were made at the beginning of the experiment and at intervals of 30 days.

The data on the normal growth of Holstein and Jersey females in weight and height as determined by Eckles [4] at this station were used as a standard of growth. Wherever "normal growth" is spoken of in the following discussion it refers to these data.

In all, 30 calves were used in these studies. A summary of the results obtained is given in Tables I to XII. While the two lots were fed slightly different rations, the difference in the results is not significant.

As will be seen from Table I, the gain in weight made by Lot I during the experiment (calves at 2 to 6 months of age) was 64 per cent of the normal gain in weight, while the increase in height during the same period was 72 per cent of the normal increase. Table II shows that Lot II made slightly larger gains in weight, averaging 68 per cent of the normal gain in weight and slightly smaller gains in height, averaging 69 per cent of the normal increase in height.

[4] ECKLES, C. H. THE NORMAL GROWTH OF DAIRY CATTLE. Mo. Agr. Exp. Sta. Research Bul. 36, 90 p., 5 fig. 1920.

In Tables III and IV the data for all calves are summarized by breeds. It will be seen that the Holstein calves on the average thrive somewhat better and make larger gains than do Jersey calves by this method of feeding.

During the first 60 days of the experiment the calves gradually became accustomed to the change in the ration, and made better gains dur-

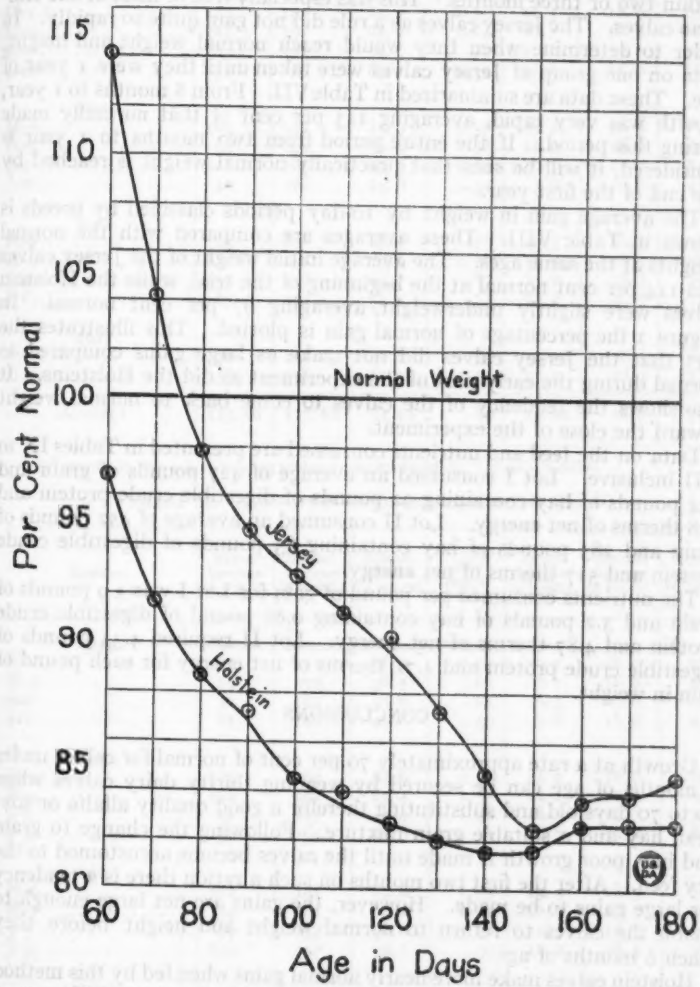

Fig. 1.—The percentage of normal gain made by Jersey and Holstein calves between the ages of 60 and 180 days.

ing the latter part of the experiment. The experimental periods were therefore divided into two 60-day periods with the results shown in Tables V and VI. During the last 60 days both lots made about 75 per cent of the normal gain in weight. But for a few animals which did very

poorly throughout the experiment, the average gain in weight would have been considerably higher. It is significant to note that about 30 per cent of the calves made normal gains during this latter period.

While all of the calves were below normal at the end of the experiment (or when they were 6 months old), many of the calves were gaining so rapidly that there was every evidence that they would become normal within two or three months. This was especially true of most of the Holstein calves. The Jersey calves as a rule did not gain quite so rapidly. In order to determine when they would reach normal weight and height, data on one group of Jersey calves were taken until they were 1 year of age. These data are summarized in Table VII. From 8 months to 1 year, growth was very rapid, averaging 123 per cent of that normally made during this period. If the entire period from two months to 1 year is considered, it will be seen that practically normal weight is reached by the end of the first year.

The average gain in weight by 10-day periods classified by breeds is shown in Table VIII. These averages are compared with the normal weights at the same ages. The average initial weight of the Jersey calves was 114 per cent normal at the beginning of the trial, while the Holstein calves were slightly underweight, averaging 97 per cent normal. In Figure 1 the percentage of normal gain is plotted. This illustrates the fact that the Jersey calves did not make as large gains compared to normal during the early part of the experiment as did the Holsteins. It also shows the tendency of the calves to come back to normal weight toward the close of the experiment.

Data on the feed and nutrients consumed are presented in Tables IX to XII inclusive. Lot I consumed an average of 427 pounds of grain and 344 pounds of hay containing 92 pounds of digestible crude protein and 528 therms of net energy. Lot II consumed an average of 452 pounds of grain and 285 pounds of hay containing 85 pounds of digestible crude protein and 517 therms of net energy.

The nutrients consumed per pound of gain for Lot I was 3.9 pounds of grain and 3.2 pounds of hay containing 0.86 pound of digestible crude protein and 4.87 therms of net energy. Lot II required 4.33 pounds of digestible crude protein and 4.71 therms of net energy for each pound of gain in weight.

CONCLUSIONS

Growth at a rate approximately 70 per cent of normal for calves under 6 months of age can be secured by weaning thirfty dairy calves when 60 to 70 days old and substituting therefor a good quality alfalfa or soybean hay and a suitable grain mixture. Following the change to grain and hay, poor growth is made until the calves become accustomed to the dry feed. After the first two months on such a ration there is a tendency for large gains to be made. However, the gains are not large enough to enable the calves to return to normal weight and height before they reach 6 months of age.

Holstein calves make more nearly normal gains when fed by this method than do Jerseys. With few exceptions, the Holstein calves reach normal weight by the eighth or ninth month, while the Jerseys reach approximately normal weight by the age of one year.

The amount of grain and hay consumed by dairy calves is an excellent indication of the rate at which growth is made.

TABLE I.—*Summary of weight and height records, Lot I*

Number.	Actual increase in weight.	Normal increase in weight.	Normal increase in weight.	Average daily gain in weight.	Actual increase in height.	Normal increase in height.	Normal increase in height.
1919.	*Pounds.*	*Pounds.*	*Per cent.*	*Pounds.*	*Cm.*	*Cm.*	*Per cent.*
H-1 [a]	162	192	84.4	1.35	12.5	18.9	66.1
H-4	124	192	64.6	1.04	16.7	18.9	88.4
415	85	161	52.8	.71	11.0	19.0	57.9
420	134	161	83.2	1.12	12.6	19.0	66.3
1920.							
H-1	165	192	85.9	1.38	14.2	18.9	75.1
H-3	94	192	48.9	.78	10.0	18.9	52.9
J-1	125	161	77.6	1.04	16.0	19.0	84.2
J-2	40	161	24.8	.33	7.0	19.0	41.1
1921.							
H-8	93	191	48.7	.78	19.3	18.3	105.5
H-9	151	191	79.1	1.26	17.0	18.3	97.4
J-7	100	157	63.7	.83	13.1	18.5	70.8
J-8	85	157	54.1	.71	13.2	18.5	71.4
1922.							
H-11	78	191	40.8	.65	11.2	18.3	61.2
H-13	158	191	82.7	1.32	13.5	18.3	73.8
Average	113.86	177.86	64.02	.95	13.44	18.7	71.87

[a] "H" as used in this and succeeding tables represents Holstein; "J," Jersey calves.

TABLE II.—*Summary of weight and height records, Lot II*

Number.	Actual increase in weight.	Normal increase in weight.	Normal increase in weight.	Average daily gain in weight.	Actual increase in height.	Normal increase in height.	Normal increase in height.
1919.	*Pounds.*	*Pounds.*	*Per cent.*	*Pounds.*	*Cm.*	*Cm.*	*Per cent.*
H-2	113	192	58.9	0.94	11.5	18.9	60.8
H-3	74	192	38.5	.62	7.7	18.9	40.7
400	30	161	18.6	.25	6.5	19.0	34.2
422	61	146	41.8	.55	7.8	17.5	44.6
1920.							
H-2	163	192	84.6	1.36	15.2	18.9	80.4
H-4	164	192	85.4	1.37	15.2	18.9	80.4
J-3	52	117	44.4	.58	8.4	14.6	54.8
J-4	132	161	81.9	1.10	16.7	19.0	87.9
1921.							
H-6	160	191	83.8	1.33	14.5	18.3	79.2
H-7	134	191	70.2	1.12	16.7	18.3	91.3
J-5	91	157	58.0	.76	11.5	18.5	62.2
J-9	110	157	70.1	.92	14.2	18.5	76.8
1922.							
H-10	172	191	90.1	1.43	15.7	18.3	85.8
H-12	158	191	82.7	1.32	14.2	18.3	77.6
H-14	153	191	80.1	1.28	15.2	18.3	83.6
J-10	136	162	84.0	1.13	11.0	17.5	62.8
Average	118.94	174.0	68.36	1.00	12.63	18.23	69.28

TABLE III.—*Summary of weight and height records of Holstein calves*

Number.	Actual increase in weight.	Normal increase in weight.	Normal increase in weight.	Average daily gain in weight.	Actual increase in height.	Normal increase in height.	Normal increase in height.
1919.	*Pounds.*	*Pounds.*	*Per cent.*	*Pounds.*	*Cm.*	*Cm.*	*Per cent.*
H-1	162	192	84.4	1.35	12.5	18.9	66.1
H-4	124	192	64.6	1.04	16.7	18.9	88.4
H-2	113	192	58.9	.94	11.5	18.9	60.8
H-3	74	192	38.5	.62	7.7	17.5	44.6
1920.							
H-1	165	192	85.9	1.38	14.2	18.9	75.1
H-3	94	192	48.9	.78	10.0	18.9	52.9
H-2	163	192	84.6	1.36	15.2	18.9	80.4
H-4	164	192	85.4	1.39	15.2	18.9	80.4
1921.							
H-6	160	191	83.8	1.33	14.5	18.3	79.2
H-7	134	191	70.2	1.12	16.7	18.3	91.3
H-8	93	191	48.7	.78	19.3	18.3	105.5
H-9	151	191	79.1	1.26	17.0	18.3	97.4
1922.							
H-10	172	191	90.1	1.43	15.7	18.3	85.8
H-11	78	191	40.8	.65	11.2	18.3	61.2
H-12	158	191	82.7	1.32	14.2	18.3	77.6
H-13	158	191	82.7	1.32	13.5	18.3	73.8
H-14	153	191	80.1	1.28	15.3	18.3	83.6
Average	136.2	191.4	71.2	1.14	14.1	18.5	76.4

TABLE IV.—*Summary of weight and height records of Jersey calves*

Number.	Actual increase in weight.	Normal increase in weight.	Normal increase in weight.	Average daily gain in weight.	Actual increase in height.	Normal increase in height.	Normal increase in height.
1919.	*Pounds.*	*Pounds.*	*Per cent.*	*Pounds.*	*Cm.*	*Cm.*	*Per cent.*
415	85	161	52.8	0.71	11.0	19.0	57.9
420	134	161	83.2	1.12	12.6	19.0	66.3
400	30	161	18.6	.25	6.5	19.0	34.2
422	61	146	41.8	.55	7.8	17.5	44.6
1920.							
J-1	125	161	77.6	1.04	16.0	19.0	84.2
J-2	40	161	24.8	.33	7.8	19.0	41.1
J-3	52	117	44.4	.58	8.4	15.6	54.8
J-4	132	161	81.9	1.10	16.7	19.0	87.9
1921.							
J-5	91	157	58.0	.76	11.5	18.5	62.2
J-7	100	157	63.7	.83	13.1	18.5	70.8
J-8	85	157	54.1	.71	13.2	18.5	71.4
J-9	110	157	70.1	.92	14.2	18.5	76.8
1922.							
J-10	136	162	84.0	1.13	11.0	17.5	62.8
Average	90.8	155.4	58.5	.77	11.45	18.3	62.6

TABLE V.—*Comparison of gains made during first and second 60-day periods of Lot I*

Number.	First 60 days.			Second 60 days.			Average daily gain in weight.
	Average daily gain in weight.	Normal gain in weight.	Normal gain in height.	Average daily gain in weight.	Normal gain in weight.	Normal gain in height.	
1919.	*Pounds.*	*Per cent.*	*Per cent.*	*Pounds.*	*Per cent.*	*Per cent.*	*Pounds.*
H-1	1.06	69.6	52.0	1.63	98.0	82.0	1.35
H-4	.82	53.3	70.0	1.25	75.0	108.9	1.04
415	.68	59.4	43.4	.73	47.8	71.4	.71
420	.92	79.7	44.6	1.32	85.9	86.7	1.12
1920.							
H-1	.97	44.6	64.0	1.78	100.7	87.6	1.38
H-3	.38	25.0	23.0	1.18	71.0	86.5	.78
J-1	.67	58.0	76.1	1.42	92.4	91.8	1.04
J-2	.12	10.1	25.0	.55	35.9	56.1	.33
1921.							
H-8	.27	23.2	61.6	1.18	73.9	157.1	.78
H-9	1.00	63.2	77.8	1.52	94.8	110.7	1.26
J-7	.67	54.8	68.4	1.00	71.4	73.3	.83
J-8	.42	34.3	81.1	1.00	71.4	61.1	.71
1922.							
H-11	.73	46.3	58.6	.57	35.4	64.3	.65
H-13	1.00	63.2	73.7	1.63	102.1	73.8	1.32
Average	.69	48.9	58.5	1.20	75.4	86.5	.95

TABLE VI.—*Comparisons of gains made during first and second 60-day periods of Lot II*

Number.	First 60 days.			Second 60 days.			Average daily gain in weight.
	Average daily gain in weight.	Normal gain in weight.	Normal gain in height.	Average daily gain in weight.	Normal gain in weight.	Normal gain in height.	
1919.	*Pounds.*	*Per cent.*	*Per cent.*	*Pounds.*	*Per cent.*	*Per cent.*	*Pounds.*
H-2	0.90	58.7	49.0	0.98	98.0	74.2	0.94
H-3	.55	35.9	24.0	.41	41.0	59.5	.62
400	.28	33.3	31.5	.12	7.6	36.7	.25
422	.53	44.9	51.1	.60	39.0	37.3	.55
1920.							
H-2	1.00	65.2	53.0	1.72	103.0	111.3	1.36
H-4	1.05	68.5	77.0	1.68	101.0	84.3	1.37
J-3	.50	43.5	60.9	.73	45.8	51.9	.58
J-4	.93	81.2	82.6	1.27	92.6	93.9	1.10
1921.							
H-6	1.40	88.4	93.9	1.26	79.2	61.9	1.33
H-7	.98	62.1	90.9	1.25	78.1	91.7	1.12
J-5	.85	69.9	65.3	.67	47.6	58.9	.76
J-9	.63	50.7	67.4	1.22	86.9	86.7	.92
1922.							
H-10	1.33	84.2	90.9	1.53	95.8	79.8	1.43
H-12	1.02	64.2	71.7	1.62	101.0	84.5	1.32
H-14	1.05	66.3	78.8	1.50	93.8	89.3	1.28
J-10	1.15	84.1	66.0	1.12	83.8	58.7	1.13
Average	.88	62.6	65.9	1.11	74.6	72.5	1.00

TABLE VII.—*Summary of weight and height records*

FROM 8 MONTHS TO 1 YEAR.

Number.	Actual increase in weight.	Normal increase in weight.	Normal increase in weight.	Average daily gain in weight.	Actual increase in height.	Normal increase in height.	Normal increase in height.
	Pounds.	*Pounds.*	*Per cent.*	*Pounds.*	*Cm.*	*Cm.*	*Per cent.*
J–7	102	116	87.93	0.95	8.9	8.5	104.71
J–8	178	116	153.45	1.49	9.2	8.5	108.24
J–5	131	116	112.93	1.10	7.5	8.5	88.23
J–9	163	116	140.51	1.36	10.4	8.5	122.35
Average	143.5	116	123.71	1.23	9.0	8.5	105.88

FROM 2 MONTHS TO 1 YEAR

J–7	280	339	82.6	0.95	28.5	32.1	88.8
J–8	374	339	110.3	1.26	27.7	32.1	86.3
J–5	308	339	90.9	1.07	24.5	32.1	76.3
J–9	373	330	110.0	1.27	28.8	32.1	89.7
Average	334	339	98.5	1.14	27.4	32.1	85.3

TABLE VIII.—*Average gain in weight*

10-day period.	Jersey.			Holstein.		
	Live weight average.	Normal gain in weight, average age 66 days.	Normal weight.	Live weight average.	Normal gain in weight, average age 67 days.	Normal weight.
	Pounds.	*Pounds.*	*Per cent.*	*Pounds.*	*Pounds.*	*Per cent.*
Start	126.6	111	114.05	161.8	167	96.8
1	128.0	123	104.06	166.0	181	91.7
2	132.1	135	97.8	173.1	195	88.7
3	138.0	146	94.5	183.8	211	87.1
4	145.0	157	92.8	192.8	228	84.5
5	154.2	169	91.3	205.4	244	84.1
6	165.4	183	90.3	216.9	262	83.7
7	173.8	199	87.3	229.0	279	82.0
8	182.0	215	84.6	242.1	297	81.5
9	188.8	229	82.4	255.5	313	81.6
10	201.7	241	83.6	271.8	329	82.6
11	213.1	254	83.8	284.1	344	82.5
12	226.0	267	84.6	298.0	360	82.7

TABLE IX.—*Summary of total feed and nutrients consumed, Lot I*

Number.	Milk.	Grain.	Hay.	Digestible crude protein.	Net energy.	Average daily gain.
1919.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Therms.*	*Pounds.*
H-1	36	628.8	496.9	134.34	770.97	1.35
H-4	36	485.8	249.5	88.06	523.60	1.04
415	34	495.7	220.9	85.92	516.24	.71
420	28	510.5	247.3	90.80	542.25	1.12
1920.						
H-1	56	501.5	395.8	107.09	614.63	1.38
H-3	56	274.3	351.3	74.34	408.38	.78
J-1	72	425.8	367.0	94.54	538.42	1.04
J-2	189	193.5	219.0	49.08	272.69	.33
1921.						
H-8	42	390.9	339.9	87.12	495.81	.78
H-9	74	501.2	453.1	113.76	645.10	1.26
J-7	56	415.1	366.7	93.21	529.71	.83
J-8	56	287.3	344.6	75.14	415.29	.71
1922.						
H-11	56	370.4	309.6	81.12	463.23	.65
H-13	56	502.5	459.7	114.69	649.71	1.32
Average	60.5	427.36	344.37	92.09	527.57	.95

TABLE X.—*Summary of total feed and nutrients consumed, Lot II*

Number.	Milk.	Grain.	Hay.	Digestible crude protein.	Net energy.	Average daily gain.
1919.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Therms.*	*Pounds.*
H-2	36	528.3	159.4	81.35	512.23	0.94
H-3	34	453.3	62.6	61.95	402.27	.62
400	34	304.2	46.7	41.06	272.32	.25
422	28	333.3	61.8	49.15	317.47	.55
1920.						
H-2	70	507.3	292.8	93.02	564.98	1.36
H-4	70	480.8	373.0	98.19	584.76	1.37
J-3	40	255.5	233.5	45.32	277.37	.58
J-4	40	433.3	259.5	80.30	487.43	1.10
1921.						
H-6	30	560.5	422.6	113.98	675.43	1.33
H-7	40	485.6	423.9	104.30	615.80	1.12
J-5	630	343.3	207.8	64.24	387.31	.76
J-9	56	444.0	324.1	88.52	529.54	.92
1922.						
H-10	56	556.9	524.7	123.56	725.18	1.43
H-12	56	501.8	457.4	109.70	645.48	1.32
H-14	56	524.5	483.7	115.26	677.58	1.28
J-10	56	521.1	324.8	98.00	592.82	1.13
Average	83.25	452.18	284.89	85.49	516.75	1.00

TABLE XI.—*Summary of food and nutrients consumed for each pound of gain, Lot I*

Number.	Milk.	Grain.	Hay.	Digestible crude protein.	Net energy.
1919.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Therms.*
H–1	0.222	3.881	3.067	0.829	4.759
H–4	.290	3.917	2.012	.710	4.223
415	.400	5.831	2.599	1.011	6.073
420	.209	3.809	1.845	.677	4.047
1920.					
H–1	.339	3.039	2.398	.649	3.725
H–3	.596	2.918	3.737	.791	4.344
J–1	.576	3.406	2.936	.756	4.307
J–2	4.725	4.838	5.475	1.227	6.817
1921.					
H–8	.451	4.203	3.655	.937	5.331
H–9	.390	3.319	3.001	.830	4.272
J–7	.560	4.151	3.667	.932	5.297
J–8	.659	3.380	4.054	.884	4.886
1922.					
H–11	.717	4.748	3.969	1.039	5.938
H–13	.354	3.180	2.909	.725	4.112
Average	.749	3.901	3.237	.857	4.867

TABLE XII.—*Summary of food and nutrients consumed for each pound of gain, Lot II*

Number.	Milk.	Grain.	Hay.	Digestible crude protein.	Net energy.
1919.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Therms.*
H–2	0.318	4.675	1.410	0.720	4.533
H–3	.459	6.127	.846	.837	5.436
400	1.133	10.140	1.557	1.369	9.077
422	.459	5.463	1.012	.806	5.204
1920.					
H–2	.429	3.112	1.796	.571	3.460
H–4	.427	2.931	2.274	.599	3.565
J–3	.769	4.913	2.567	.871	5.334
J–4	.303	3.281	1.966	.608	3.693
1921.					
H–6	.188	3.503	2.641	.712	4.221
H–7	.299	3.631	3.163	.778	4.596
J–5	6.023	3.772	2.284	.706	4.256
J–9	.509	4.036	2.946	.805	4.814
1922.					
H–10	.325	3.237	3.050	.718	4.216
H–12	.354	3.175	2.894	.697	4.085
H–14	.365	3.428	3.161	.753	4.428
J–10	.411	3.831	2.388	.721	4.358
Average	.854	4.328	2.247	.767	4.705

XVI, No. 9

Lot I

Net energy.

Therms.
4. 759
4. 223
6. 073
4. 047

3. 725
4. 344
4. 307
6. 817

5. 331
4. 272
5. 297
4. 886

5. 938
4. 112

4. 867

, Lot II

Net energy.

Therms.
4. 533
5. 436
9. 077
5. 204

3. 460
3. 565
5. 334
3. 693

4. 221
4. 596
4. 256
4. 814

4. 216
4. 085
4. 428
4. 358

4. 705